v2, 837, (Year Book Publishers, Chicago)
9. Radford, E. P., Jr., Lefcoe, N., and Mead, J., *Fed. Proc.*, 1954, v13, 114.
10. von Neergaard, K., *Z. f. d. ges exp. Med.*, 1929, v66, 373.
11. Lenard, P., v. Dallwitz-Wegener, R., and Zachmann, E., *Ann. Physik.*, 1924, v74, 381.
12. Harkins, W. D., and Jordan, H. F., *J. Am. Chem. Soc.*, 1930, v52, 1751.
13. Harkins, H. N., and Harkins, W. D., *J. Clin. Invest.*, 1929, v7, 263.
14. duNouy, P. L., *Surface Equilibria of Biological and Organic Colloids*, Am. Chem. Soc. Monograph No. 27 (Chemical Catalogue Co., New York, 1926).
15. McIlroy, M. B., *Thorax*, 1952, v7, 281.

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Metabolism of Benzoic Acid in Normal and Irradiated Rats.*† (21285)

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In a previous communication, it has been shown that the "free glycine pool" increases in size after rats have been exposed to total body x-radiation(1). These conclusions were based on determinations, before and after irradiation, of the isotope concentration of the urinary hippuric acid excreted after injection of C-14 labeled glycine and benzoic acid. The experimental technic was modified somewhat to test the ability of irradiated rats to conjugate labeled benzoic acid. The conjugation process may be looked upon as a 2-step process involving coenzyme A (Co A) and transesterification at the S-acyl bond(2).

Benzoic acid + acetyl — Co A→Benzoyl-Co A + acetic acid(1)

Benzoyl-Co A + glycine→Benzoyl glycine + Co A(2).

Studies with benzoic acid-carboxyl-C^{14} revealed that radiation did not interfere with hippuric acid synthesis. It was noted also that a marked dilution of the specific activity of urinary hippuric acid occurred in starved animals and this suggested that there must be an endogenous source of benzoic acid. The findings to be reported in this paper indicate that benzoic acid may be the end product of an as yet little explored metabolic pathway of one of the aromatic amino acids, *e.g.*, phenylalanine.

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Experimental. Four of 8 Wistar-type rats, all weighing 150-175 g, were exposed to 700 roentgens of x-rays. Immediately after the irradiation procedure, all were given an intraperitoneal injection of sodium benzoate-carboxyl-C^{14} followed by 2 more injections at 24-hour intervals. The injected solutions contained 18.3 mg of sodium benzoate. The specific activity of the material used in the initial dose was 22,100 c.p.m. per mg carbon and that used in each of the 2 succeeding doses was 18,000 c.p.m. per mg carbon. The rats were placed in confining metabolism cages and urine (without fecal contamination) was collected at daily intervals. The animals were not fed during the 3-day period of study and were not given water except for the 10 ml of saline in which each daily dose of sodium benzoate was dissolved. A description of the animals and irradiation procedure used is given elsewhere(1). The total amount and the specific C^{14} activity of the urinary hippuric acid was determined according to the method of Gaffney and coworkers(3). The C^{14}-activity of the unconjugated urinary benzoic acid was determined by measuring the radioactivity of the appropriate spot on the paper chromatograms used for the assay of hippuric acid. The total quantity of the excreted benzoic acid was not determined chemically.

Results. The data obtained are presented in Table I. Average values as well as ranges

TABLE I. Incorporation of Benzoic Acid-Carboxyl-C^{14} into N-Benzoyl Glycine.

Collection period after isotope admin.		Urinary excretion of total C^{14} activity admin. As hippuric acid, %	As benzoic acid, %	Micromoles of hippuric acid excreted	C^{14} activity* of hippuric acid	Expected C^{14} activity of hippuric acid	RIC†
		(1)	(2)	(3)	(4)	(5)	(6)
1st 24 hr	Irradiated	75.9	2.53	108.5	10800	17400	.49
		70.7–84.2	1.37– 3.06	104.9–111.6	10287–11073		.46–.50
	Control	64.4	6.60	104.5	9580	"	.43
		59.4–71.6	3.43–10.73	102.7–106.6	8647–10688		.39–.48
2nd 24 hr	Irradiated	58.8	8.02	105.3	7060	14000	.39
		51.5–67.2	5.92–10.74	102.1–103.8	6528– 7866		.36–.44
	Control	76.9	7.06	105.3	8930	"	.50
		62.2–85.0	4.82– 9.16	102.1–108.8	7713–10156		.43–.56
3rd 24 hr	Irradiated	61.2	7.86	97.5	8140	"	.45
		51.7–76.7	5.62–10.19	92.1–105.5	7633– 9216		.42–.51
	Control	74.6	8.67	99.9	9471	"	.53
		66.6–83.9	6.36–11.58	96.5–104.4	8653–10953		.48–.61

* C^{14}-activity = Counts/min./mg carbon.

† Relative isotope conc. = $\frac{\text{c.p.m./mg carbon of hippuric acid isolated}}{\text{c.p.m./mg carbon of benzoic acid injected}}$.

of the experimental and control animals are shown in columns 1-4 and 6. For purposes of comparison with column 4, the expected isotope concentration of the hippuric acid is given in column 5. This theoretical value was calculated on the assumption that the introduction of the 2 inert carbon atoms of the glycyl residue (*cf.* reaction 2) constitutes the only dilution of the C^{14}-activity. The fact that the labeled benzoate is not excreted quantitatively has not been taken into consideration.

Evaluation of the data in Table I shows that radiation exposure has no apparent effect on the ability of rats to conjugate benzoic acid. Although the total amount of hippuric acid excreted by the irradiated and control rats was not altered during the period of study, the isotope concentration of the hippuric acid excreted by irradiated rats is somewhat less than that of the controls of the second day.

Of more interest is the observation that there is far greater dilution of the specific C^{14}-activity of hippuric acid than could be accounted for, except by postulating a source of benzoic acid in addition to that administered. The fact that the isotope dilution does not fall during a three day period of starvation suggests that food is unlikely to be the unknown source of benzoic acid.

It is of interest to mention other observations made in relation to benzoic acid metabolism. Subsequent experiments have indicated (a) that rats on a benzoic acid-free diet continue to excrete hippuric acid in amounts comparable to that excreted when they are on a Purina fox chow diet. (b) Hippuric acid has been isolated from the urine of rats on a high aureomycin, benzoic-acid-free diet as well as (c) in urine samples excreted by rats on the second day after complete removal of the alimentary tract. It has been observed (d) that hippuric acid is present in the second urine sample excreted after birth by human babies(4).

Discussion. It has been mentioned that these studies were undertaken to ascertain whether radiation exposure impairs the ability of rats to conjugate benzoic acid. It seems clear from the data that there is no serious interference with benzoic acid conjugation during the early post-irradiation period demonstrable under these conditions of irradiation and testing. During this early period after exposure to x-rays changes in the "free-glycine" pool have been observed(1).

The observation of the dilution of the injected labeled benzoic acid by a factor of 1.5-2.0 even in the starved rats was not an entirely unexpected finding since it has been observed for some time that animals excrete

hippuric acid even when benzoic acid has not been administered. Although certain forms of bacteria are known to have the ability to produce benzoic acid, this substance has not been described as a normal metabolite in vertebrates. Current isotope studies together with the observations reported in this paper provide evidence for believing that there is an endogenous source of benzoic acid which is not dependent upon food intake or on the activity of intestinal bacteria. The most likely precursor is one of the aromatic acids and, indeed, preliminary studies suggest that phenylalanine is one of the amino acids involved(5).

Summary. 1. The isotope concentration of the urinary hippuric acid of starved irradiated or non-irradiated rats given benzoic acid-carboxyl-C^{14} is much less than the dilution expected from the conjugation of the injected labeled benzoic acid with inert carbon atoms of the glycyl residue. 2. Evidence is presented that benzoic acid is a normally occurring metabolite in rats. 3. The effect of x-radiation on the ability of starved rats to conjugate benzoic acid-carboxyl-C^{14} has been tested and no defect detected under the experimental conditions used.

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1. Schreier, K., DiFerrante, N., Gaffney, G. W., Hempelmann, L. H., and Altman, K. I., *Arch. Biochem. Biophys.*, 1954, v50, 417.

2. Schachter, D., and Taggart, J. V., *J. Biol. Chem.*, 1953, v203, 925.

3. Gaffney, G. W., Schreier, K., DiFerrante, N., and Altman, K. I., *ibid.*, 1954, v206, 695.

4. Schreier, K., unpublished observations.

5. Bruns, F., Haberland, G. L., and Altman, K. I., experiments to be published.

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Action of Poly-α-Amino Acids on Clotting of Fibrinogen by Thrombin.* (21286)

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It was shown previously that basic polyamino acids inhibit blood thromboplastic activity(1), fibrinolysis(2), proteolytic activity of pepsin(3), clearing of alimentary lipemia by heparin(4), as well as bacterial(5) and viral growth(6). Acidic polyamino acids neutralized the inhibitory actions of basic polyamino acids.

In this communication it is reported that the basic polyamino acids, polylysine, polyornithine and polyarginine, accelerated the clotting of fibrinogen by thrombin, while the acidic polyamino acids, polyaspartic, polyglutamic and polycysteic acids retard the clotting.

Materials and methods. Fibrinogen. Bovine Fraction I† was used or purified by the procedure of Laki(7). The final preparation contained 88-93% clottable protein and was electrophoretically homogenous. Mobility calculated from the descending boundary pattern was 1.8×10^{-5} cm^2 V^{-1} $sec.^{-1}$ at pH 7.4, ionic strength 0.15 and t = 4°C. Determinations of fibrinogen were made by the procedure of Ware *et al.*(8).

Thrombin. A thrombin solution was obtained by adding 19 ml of isotonic buffer solution (see below) and 1 ml of 0.1 M sodium oxalate to a 1000 unit vial of Bovine Thrombin (Upjohn). One g of $BaSO_4$ (Merck) was added, the suspension was stirred for 15 minutes, centrifuged and the sediment discarded. This treatment reduced the protein content

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