Free Porphyrin Concentrations and Porphyrin Synthesis in Duck Tissues* (32660)

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Animal tissues normally contain free, i.e., nonheme, porphyrins. This is well known although quantitative data appear limited. Findings for erythrocytes of various species (1), rat liver, kidney, and spleen (2), human liver (3), and pig nerve (4) suggest a roughly tenfold range of protoporphyrin concentration and a lesser range of coproporphyrin concentration in tissues of animals in general. The significance of the free porphyrin in tissues and of differences in concentration is obscure. In the present work, to provide a basis for inferences about significance, the concentrations of free porphyrin in a number of tissues of the mature, male Pekin duck (*Anas domesticus*) were measured. Tests were also made for incorporation of glycine-2-^{14}C into free porphyrin and heme by the tissues *in vitro*.

Experimental. Animals weighing about 3 kg each were obtained from a local farm, kept 2 or more weeks on a usual commercial ration, fasted 18 hours, and then decapitated. Intestine between pylorus and cecae, portions of gizzard striated muscle, breast muscle, and liver, and certain whole organs were promptly collected. Blood was collected in heparin. The lumen of the sample of intestine was washed with water before further treatment. Tissues for porphyrin and heme assay were frozen in liquid nitrogen immediately after collection and stored frozen until used. Tissues for tests of glycine incorporation were cooled in ice and used not more than one-half hour after collection.

Porphyrin and heme were extracted from tissue with an ethyl acetate–acetic acid mixture (5). The extract was washed with water. No porphyrin was detected in the washing from any tissue; thus, the tissues presumably did not contain uroporphyrin (6). The porphyrin in the washed ethyl acetate was transferred to 3 *N* HCl and then to ethyl ether. Coproporphyrin was removed from the ether with 0.1 *N* and protoporphyrin subsequently with 3 *N* HCl (5). Coproporphyrin was transferred from the dilute acid to ether and then to 3 *N* acid. Where indicated, the total porphyrin, i.e., copro- plus protoporphyrin, was transferred from the ether to 3*N* acid. The final acid solutions were assayed for porphyrin fluorometrically (1) and examined spectrophotometrically for wavelengths of maximum light absorption in the Soret region. The tissue proto- and coproporphyrin preparations absorbed light maximally at 409–410 and 402–403 mμ, respectively. Maxima for comparable solutions of authentic proto- and coproporphyrin were observed at 410 and 403 mμ, respectively. The absorption peaks of the tissue preparations were not as sharp as those of the authentic materials. Thus, as is generally the case for porphyrin isolated from biological materials as indicated (1), the preparations probably contained more than one porphyrin.

After porphyrin removal the ethyl acetate extract and appropriate solutions of hemin were compared in the Evelyn colorimeter using filter 400 or in the spectrophotometer at 380 mμ, a wavelength at which light was absorbed maximally. For all tissues, the estimate of heme provided by the comparison was practically equivalent to that indicated by iron assay of the extract and, in the case of blood, by oxyhemoglobin assay. The heme extracted from the tissues derived from heme–protein complexes linked by noncovalent bonds, e.g., hemoglobin.

For test of labeled glycine incorporation into porphyrin and heme, solid tissues were sliced and added to a roughly equal weight of 0.01 *M* glucose in Ringer's bicarbonate solution in the cold. The tissue preparations and blood were incubated with about 1 μC of glycine-2-^{14}C and 100 mμmoles of total added glycine per gm of preparation and blood for 3 hours under 5% CO_2 in O_2 and then assayed for amount and specific activity of

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the total porphyrin and heme (7). Hemin was added as carrier to ethyl acetate extracts of solid tissues to facilitate isolation of heme for the determination of specific activity. Glycine and ^{14}C in protein-free extracts of washed incubated tissues were also determined (7). For each tissue, comparison was made between the ratio of ^{14}C to glycine in an extract and the specific activity of formaldehyde derived from the α-carbon of the glycine (7). The ratios for liver and other tissues, respectively, were about 6 and 0–3% higher than corresponding formaldehyde activity. Thus, as for erythrocytes (7), the ratios are suitable estimates of the specific activities of the glycine in the tissues. For each tissue, comparison was made between the specific activities of total porphyrin and protoporphyrin. The activities differed by less than 10% and thus may be considered equal.

Results and Discussion. Results are summarized in Table I. The erythrocyte porphyrin concentrations were calculated from those for blood, since erythrocytes contain most of the porphyrin in blood (1, 5, 7). Solid tissues obtained as indicated contain trapped erythrocytes. Adjustment for such erythrocytes based on the heme concentrations decreases the porphyrin concentrations listed for kidney and spleen by about one third and for other tissues by one tenth or less. As evident from the heme specific activities, the adjustment provides an exaggerated estimate of the erythrocyte porphyrin in some tissues. The ratio of proto- to coproporphyrin ranged from about 5 in liver to 22 in erythrocytes. The proto- and coproporphyrin concentrations listed are not significantly correlated ($p > .1$). Presumably, therefore, the concentrations of the porphyrins in tissue are independently controlled. No connection between the porphyrin concentrations and known functional activities of the tissues is apparent.

The heme specific activities for gizzard, heart, and breast muscle are lower than corresponding erythrocyte activities. Thus, the several muscles, as opposed to erythrocytes trapped therein, may have contained no labeled heme. The heme specific activities for the other tissues indicate the presence of labeled heme. The porphyrin specific activities listed, when adjusted for trapped erythrocytes,

TABLE I. Porphyrin and Heme Concentrations in Fresh Duck Tissues and Specific Activities of Porphyrin, Heme, and Glycine in Tissues Incubated with Glycine-2-^{14}C.[a]

Tissue	Concentrations				Specific activities				
	Protoporphyrin ($m\mu$moles/10^3 gm)		Coproporphyrin ($m\mu$moles/10^3 gm)		Proto- plus coproporphyrin ($m\mu$moles/10^3 gm)		Heme ($m\mu$moles/10^{-1} gm)		Total porphyrin (cpm/$m\mu$mole)		Heme (cpm/$m\mu$mole)		Glycine (cpm/8 $m\mu$moles)	
Erythrocytes	1200	*20*	55	*40*	1200	*18*	1600	*4*	170	*14*	4	*25*	1360	*7*
	(7)		(7)		(15)		(15)		(6)		(6)		(6)	
Liver	500	*24*	100	*30*	670	*22*	51	*26*	76	*32*	180	*35*	340	*5*
	(4)		(4)		(13)		(12)		(5)		(5)		(5)	
Heart	410	*27*	25	*50*	400	*25*	39	*32*	6	*42*	1	*50*	1500	*4*
	(4)		(4)		(13)		(10)		(4)		(4)		(4)	
Gizzard	400	*8*	51	*30*	420	*31*	42	*30*	39	*50*	1	*45*	960	*5*
	(4)		(4)		(12)		(10)		(4)		(4)		(4)	
Intestine	330	*15*	60	*47*	300	*25*	16	*28*	44	*35*	22	*43*	400	*7*
	(4)		(4)		(12)		(12)		(5)		(5)		(5)	
Kidney	250	*35*	23	*35*	240	*29*	100	*34*	41	*20*	16	*32*	530	*10*
	(5)		(5)		(12)		(13)		(6)		(5)		(6)	
Spleen	220	*14*	34	*50*	230	*15*	110	*31*	50	*18*	40	*18*	480	*10*
	(5)		(5)		(9)		(10)		(3)		(3)		(3)	
Breast muscle	170	*38*	10	*39*	130	*30*	18	*34*	25	*48*	1	*40*	1700	*20*
	(4)		(4)		(10)		(12)		(4)		(4)		(4)	
Brain	88	*22*	12	*40*	83	*22*	11	*36*	44	*36*	17	*39*	1100	*9*
	(5)		(5)		(12)		(11)		(5)		(5)		(5)	
Testes	42	*31*	3	*22*	58	*25*	6	*27*	190	*46*	220	*38*	300	*21*
	(4)		(4)		(12)		(9)		(5)		(5)		(5)	

[a] The concentrations and specific activities listed are means of results for the number of animals noted in parentheses. The italicized numbers are standard deviations expressed as percentages of the respective means. The concentrations are for fresh tissues; the specific activities for tissue slices incubated 3 hours with glycine-2-^{14}C. The glycine specific activities are observed values. The other specific activities are observed values multiplied by *glycine specific activity for erythrocytes* (1360 cpm/8 $m\mu$moles)/*glycine specific activity for tissue.*

indicate that heart and spleen, kidney, breast muscle, and other tissues, respectively, contained 0, 30, 50, and 70–100% of the labeled free porphyrin found. Since heart tissue may have contained neither labeled heme nor porphyrin, it is uncertain whether it synthesized porphyrin during the incubation. The presence of labeled heme or porphyrin or both in the other tissues indicates synthesis. Tissue porphyrin concentrations before and after incubation, as in the tests for glycine incorporation, differed by ± 10% or less. Thus, porphyrin synthesis was accompanied by little change in porphyrin concentration. In blood the porphyrin concentration usually increases slightly during incubation (7, 8). The coefficients of correlation between amounts of labeled heme and porphyrin indicated by the data in Table I and the total porphyrin concentrations are positive and significant ($p < .05$). The free porphyrin concentration in tissues in general may, therefore, be a function of the rate of porphyrin synthesis.

The ratios of labeled heme to total free porphyrin are about 0.7–1 for gizzard, heart, and breast muscle and 16 or more for other tissues. According to argument applied earlier to erythrocytes (7), the ratios indicate that the porphyrin specific activities for the several muscles and the other tissues, respectively, would have been roughly one half to one third less and equal to the corresponding glycine specific activities if the total free porphyrin had been the immediate source of labeled heme porphyrin. As found earlier for erythrocytes (7), the porphyrin specific activities were markedly less than the glycine activities. Apparently, therefore, a considerable portion of the free porphyrin in the tissues, as in erythrocytes, is not immediately available for heme formation. Conceivably, such porphyrin has a function.

Summary. Proto- and coproporphyrin concentrations in Pekin duck tissues were determined. The tissues and corresponding protoporphyrin concentrations (mμmole/kg) were erythrocytes (1200), liver (500), heart (410), gizzard (400), intestine (330), kidney (250), spleen (220), breast muscle (170), brain (88), and testes (42). The ratio of proto- to coproporphyrin concentrations ranged from 5 in liver to 22 in erythrocytes. The porphyrin concentrations were not significantly correlated. As indicated by glycine-2-^{14}C incorporation into free porphyrin, heme, or both, the tissues, except possibly heart, synthesized porphyrin *in vitro*. Correlation between porphyrin synthesis and concentration was positive and significant.

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