Biochem. J. (1964), **92**, 300

Distribution and Metabolism of DL-3,4-Dihydroxy[2-^{14}C]-phenylalanine in Rat Tissues

BY K. F. GEY AND A. PLETSCHER
Medical Research Department, F. Hoffmann–La Roche and Co. Ltd., Basle, Switzerland

(*Received* 31 *December* 1963)

L-Dopa* is generally assumed to be a precursor of catecholamines, e.g. 3-hydroxytyramine, norepinephrine and epinephrine (for reviews see Blaschko, 1959; Holtz, 1959; Kirshner, 1959; Leeper, 1959). In normal tissues and urine, however, dopa occurs in minute amounts (Carlsson & Hillarp, 1962; Dresse, 1961; Gamo, 1959; Gerritsen, Copps & Waisman, 1961; Goodall, 1951; Hall, Hillarp & Thieme, 1961; Montagu, 1957; Murphy & Sourkes, 1961; Sano *et al.* 1959; Shaw, McMillan & Armstrong, 1957; Sourkes, Murphy & Woodford, 1960; Wiegand & Perry, 1961). Although this amino acid is widely used in pharmacological experiments, its distribution and metabolism in tissues have not been investigated systematically. It has been reported that, after the injection of L- or DL-dopa, the concentration of the amino acid (as well as that of 3-hydroxytyramine) increases in the brain, lungs, kidney, heart and spleen (Goldstein, Musacchio & Gerber, 1963; Sourkes, 1961; Wegmann, Kako & Chrysohou, 1961). Further, 24 hr. after the injection of DL-[2-^{14}C]dopa, only negligible amounts of radioactivity were found in the liver, kidney, heart and spleen, but more than 80 % was recovered from the urine and 2 % from the expiratory air (Pellerin & D'Iorio, 1955, 1956/57). Recent radioautographic studies at various time-intervals after the intravenous injection of DL-[2-^{14}C]dopa showed a high overall radioactivity in the adrenal medulla, pancreas, kidney, intestinal mucosa, salivary glands, bone marrow and liver (Rosell, Sedvall & Ullberg, 1963).

The present paper deals with the tissue distribution of DL-[2-^{14}C]dopa and of its metabolites in the albino rat. Extracts of organs were fractionated into phenolic amino acids, amines and metabolic end products (e.g. phenolcarbonic acids, phenol alcohols etc., briefly designated as phenolcarbonic acids).

EXPERIMENTAL

Materials

DL-[2-^{14}C]Dopa (specific activity 3·24–9·51 mc/m-mole; 16·4–48·2 μc/mg.), obtained from The Radiochemical Centre, Amersham, Bucks., or prepared by Dr J. Würsch, F. Hoffmann–La Roche and Co. Ltd., Basle, was diluted with L-dopa to give a final L-dopa content of 87 %. 3-Hydroxy[2-^{14}C]tyramine hydrochloride (1·2 mc/m-mole) and [2-^{14}C]norepinephrine acetate (20·5 mc/m-mole) were synthesized by Dr J. Würsch, F. Hoffmann–La Roche and Co. Ltd., Basle, and the Centre d'Études Nucléaires de Saclay (France) respectively.

Methods

Treatment of animals. Female albino rats (weighing 120–130 g. and kept without food for 14–16 hr.) received DL-[2-^{14}C]dopa (20 mg./kg., in 5 ml. of N_2-saturated water/kg.) by subcutaneous injection.

Preparation of tissues. After decapitation and exsanguination of the rats, the blood was allowed to run into 1 vol. of ice-cold 7 % (w/v) $HClO_4$. The organs were then homogenized in 4 vol. of ice-cold 4 % (w/v) $HClO_4$ under CO_2, by using a motor-driven conical all-glass homogenizer (Kontes duall) (aorta, heart and intestine after previous washing with ice-cold 0·9 % NaCl). The homogenates and the blood extracts were kept at 2° for 30 min. and then centrifuged at 12 100g for 10 min. at 2°. A sample of the supernatant was titrated automatically (Titrator TTT_1; Radiometer, Copenhagen) to pH 5·0 with 2N-KOH under N_2 and with cooling. After 10 min. the crystals of $KClO_4$ were removed as above by centrifugation. To hydrolyse the *O*-conjugates (i.e. glucuronate and sulphate esters) 20 ml. of each supernatant was incubated anaerobically at 37° for 2 hr. with 0·5 ml. of dialysed glusulase (50 000 units of glucuronidase and 10 000 units of sulphatase; Endo Laboratories, Richmond Hill, N.Y., U.S.A.), supplemented with 5 mg. of EDTA, 20 mg. of ascorbic acid and 2 ml. of 0·5M-potassium acetate buffer, pH 5·0. After a second protein precipitation with $HClO_4$, the supernatant was kept at −20° overnight.

Separation on two cation-exchange columns. Samples of the supernatant were adjusted automatically to pH 6·0 with 2N-KOH at 2° under N_2, and subsequently $KClO_4$ was removed by centrifugation. The amines were adsorbed (1 drop/10 sec.) according to the procedure of Bertler, Carlsson & Rosengren (1958) on column I of 0·5 g. of Dowex 50 (X4; K^+ form; 200–400 mesh; cycled according to Hirs, Moore & Stein (1953) and finally treated with 0·5M-sodium acetate buffer, pH 6·0]. After washing column I with four 5 ml. lots of water, the amines were eluted with 20 ml. of metal-free 2N-HCl (eluate I). The amino acids were separated from the fraction containing phenolcarbonic acids, phenol alcohols etc. on column II, consisting of 0·5 g. of Dowex 50 (H^+ form). For this purpose the effluent (combined with the water washings) of column I was brought to pH 2·8 with N-HCl and allowed to run through column II which retained the amino acids. The effluent of column II containing the phenolcarbonic acids was pooled with three subsequent washings each of 2 ml. of 0·01N-HCl. Finally,

* Abbreviation: dopa, 3,4-dihydroxyphenylalanine.

the amino acids were eluted from column II with 20 ml. of 0·5M-potassium acetate buffer, pH 6·5 (eluate II). The principle of the fractionation is shown in Scheme 1.

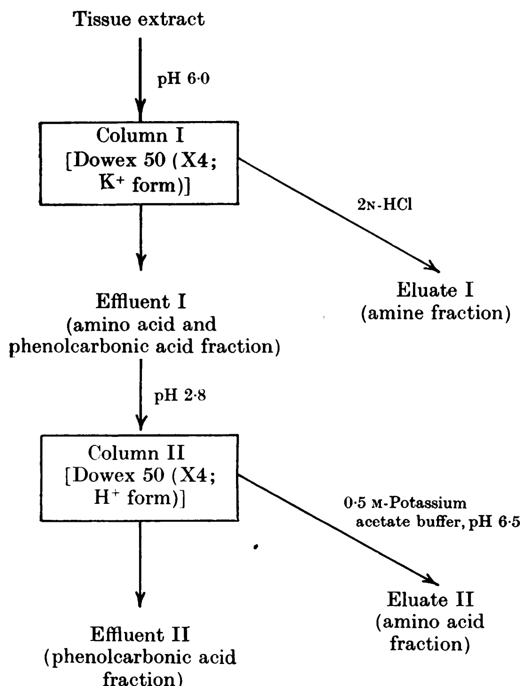
Measurement of radioactivity by liquid-scintillation counting. After drying a sample of eluate I (amines) in a rotating evaporator, the residue was dissolved in water and 1 ml. was mixed with a solution of 15 ml. of 0·7% 2,5-diphenyloxazole and 0·005% 1,4-bis-(5-phenyloxazol-2-yl)-benzene in toluene–ethanol (1:1, v/v). Samples (1 ml. of effluents I and II, which had been combined with the corresponding washings, as well as the eluate II) were added directly to 15 ml. of the toluene–ethanol solution with 2,5-diphenyloxazole and 1,4-bis-(5-phenyloxazol-2-yl)benzene. Under these conditions, eluates and effluents caused no quenching. Standard amounts of [^{14}C]dopa were counted similarly to determine the relative total radioactivity (Packard Tri-Carb scintillation spectrophotometer).

Subfractionation. (*a*) Amino acids. The amino acids adsorbed on column II were eluted by subsequent portions of 0·1N-, 0·2N-, and 0·4N-HCl and characterized by paper chromatography on Schleicher–Schüll no. 2043b paper with butanol–acetic acid–water (4:5:1, by vol.).

(*b*) Amines. Samples of eluate I were freeze-dried after the addition of 1 drop of dimercaptopropanol. The residue was dissolved in 1·0 ml. of methanol–water (9:1, v/v), with 1 drop of dimercaptopropanol/100 ml., and 0·4 ml. was spotted in duplicate on paper strips 3·0 cm. wide (Schleicher–Schüll no. 2043b, washed) by an automatically driven Agla syringe under a stream of cold N_2. The paper chromatograms were developed with butanol satd. with 0·5N-HCl for 24 hr. at 20°. One strip was then sprayed with Folin–Denis reagent, and the second was cut into pieces (0·5–1·0 cm.) for radioactivity determinations made in Tri-Carb counting vessels (Wang & Jones, 1959), containing a special centre well (diam. 1 cm.), filled with a solution of 0·4% 2,5-diphenyloxazole and 0·015% 1,4-bis-(5-phenyloxazol-2-yl)-benzene in toluene. The 3-hydroxytyramine was separated from normetanephrine by rechromatography with benzene–propionic acid–water (2:2:1, by vol.) on Whatman no. 1 paper.

(*c*) Phenolcarbonic acids. The effluent II combined with the washings was adjusted to pH 1·5 with N-HCl, saturated with NaCl and extracted three times with 2 vol. of peroxide-free ethyl ether. The residue of the ether extracts, dried with Na_2SO_4, was dissolved in 1·0 ml. of methanol–water (as above) for paper chromatography with benzene–propionic acid–water (2:2:1, by vol.) for 2½ hr. at 20°. The spots were detected with diazotized sulphanilic acid spray reagent.

(*d*) Recovery. The DL-[2-^{14}C]dopa, 3-hydroxy[2-^{14}C]-tyramine and [2-^{14}C]norepinephrine added to $HClO_4$ extracts of blood, brain and heart were obtained with recoveries of 95–100%. In animals injected with DL-[2-^{14}C]-dopa, however, the radioactivity could not be completely recovered from all tissues. Thus in the $HClO_4$ extract of the homogenates the following percentages of radioactivity, compared with values obtained by combustion (Kalberer & Rutschmann, 1961), were found: 78% in skeletal muscle; 85–92% in liver, small intestine, skin, kidney and brain; 100% in heart and adrenals. The values in the Tables are not corrected, except where stated.



Scheme 1. Principle of fractionation of three extracts. Details are given in the Experimental section.

RESULTS

Time-course in blood and brain. The overall radioactivity/ml. of blood, given as the sum of radioactivity in the three fractions, was highest 20 min. after the subcutaneous injection of DL-[2-^{14}C]dopa; it markedly decreased within the next 3 hr. and subsequently showed a rather constant decline. The amino acid fraction contained between 64 and 96% of the overall radioactivity and had a similar time-course. The radioactivities of the amine and phenolcarbonic acid fractions rose to maximal values within the first hour and dropped to zero after 9 and 27 hr. respectively. The amine fraction contained at the most 13% of the overall radioactivity (Fig. 1).

In the brain, the total overall radioactivity/g. of fresh tissue was also maximal 20 min. after the injection of DL-[2-^{14}C]dopa, but decreased less rapidly than in the blood. The radioactivity of the amino acid fraction in the brain, in contrast with that in the blood, increased slowly, with maximal values from the first to the ninth hour and representing about 50–95% of overall radioactivity. The radioactivity of the amine fraction in the brain, similar to that in the blood, was highest within the first hour and then slowly dropped to zero; this fraction contained at the most 7% of the overall radioactivity. The radioactivity of the phenolcarbonic acid fraction was 65% of the overall radioactivity 20 min. after the injection of DL-[2-^{14}C]-

dopa, 41 % after 1 hr. and then quickly dropped to lower values.

Comparison of different organs. The following order of radioactivity/g. of tissue was found in the various organs (Tables 1 and 2) 1 hr. after the subcutaneous injection of DL-[2-^{14}C]dopa:

Overall radioactivity: brain ⟨⟨ skeletal muscle ⟨ heart ~ aorta ~ blood ⩽ carcass ⟨⟨ skin ~ intestine ~ adrenals ~ liver ⟨⟨⟨ kidney.

Amino acids: brain ⟨⟨ skeletal muscle ~ aorta ~ heart ⩽ intestine ~ liver ~ blood ⩽ adrenals ~ carcass ⟨⟨ skin ⟨ kidney.

Amines: brain ⟨⟨⟨ skeletal muscle ⟨ heart ~ blood ~ aorta ⩽ skin ⟨ carcass ⟨⟨⟨ kidney ~ intestine ~ adrenals ⩽ liver.

Phenolcarbonic acids: skeletal muscle ⟨ adrenals ⩽ brain ⟨ blood ~ carcass ~ skin ⟨ heart ⟨ aorta ⟨ liver ~ intestine ⟨⟨⟨ kidney.

The following order of radioactivity per whole organ was found (Table 3) 1 hr. after the subcutaneous injection of DL-[2-^{14}C]dopa:

Overall activity: aorta ~ adrenals ⟨ heart ⟨⟨ brain ⟨⟨⟨ blood ~ kidney ⟨⟨ intestine ~ liver ⟨⟨ skeletal muscle ⟨ skin ⟨⟨⟨ carcass.

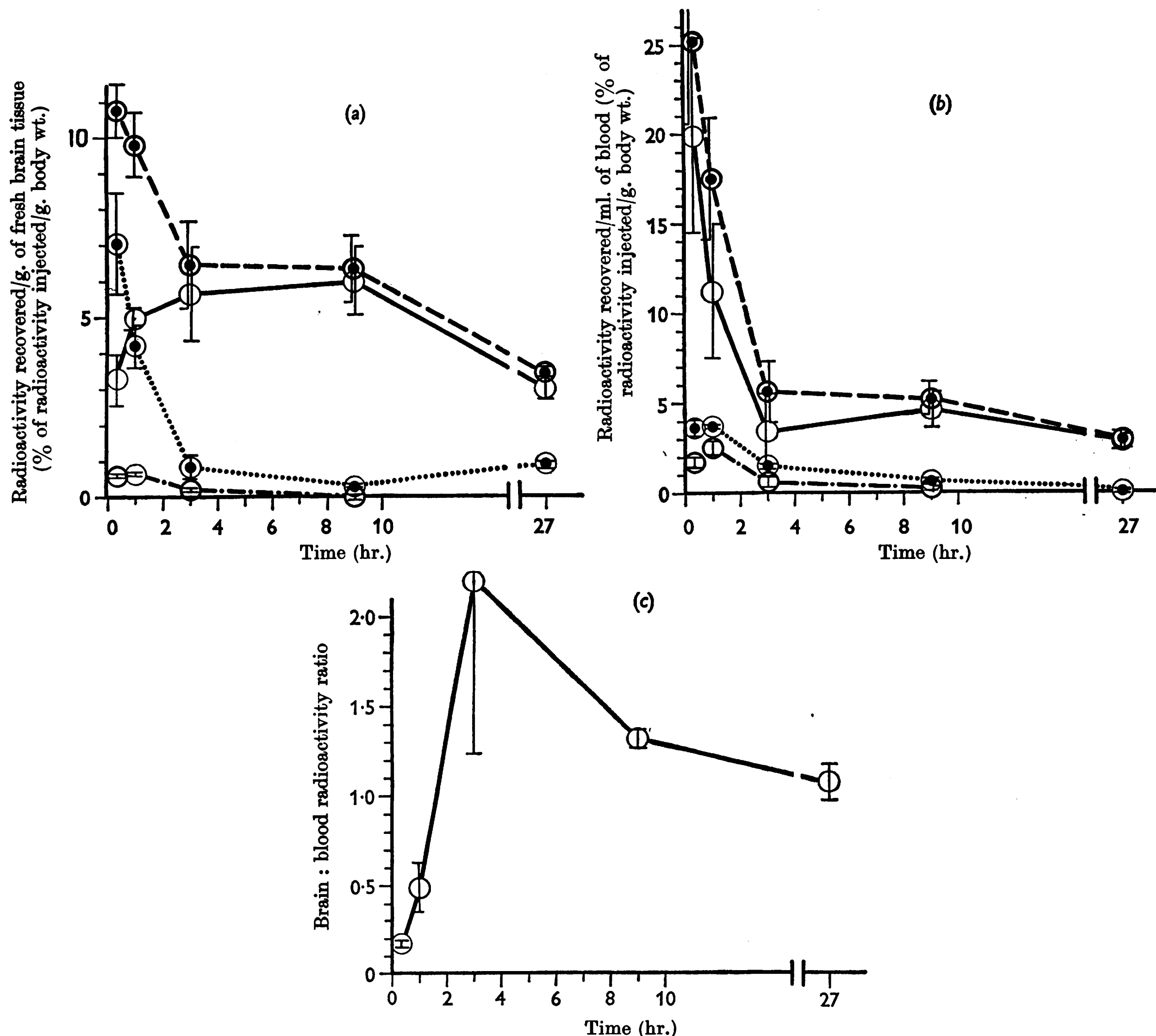


Fig. 1. Time-course of the total radioactivity as well as of the radioactivity of the various fractions after the subcutaneous injection of DL-[2-^{14}C]dopa in rats. ——, Amino acids; –·–·–·, amines; ......, phenolcarbonic acids; – – – –, overall activity. The points represent averages ±S.E.M. of two experiments with brain (*a*) and blood (*b*) each with three rats. The brain: blood ratios (*c*) were calculated separately in each experiment.

Amino acids: aorta < adrenals < heart << brain <<< kidney < intestine ~ liver < blood <<< skeletal muscle << skin < carcass.

Amines: aorta < brain ~ adrenals < heart <<< blood << kidney << skin ~ skeletal muscle <<< intestine ~ liver <<< carcass.

Phenolcarbonic acids: adrenals < aorta << heart << brain <<< blood < liver ~ intestine << skin < kidney ~ skeletal muscle <<< carcass.

The radioactivity of the carcass was higher than the sum of the radioactivity in the organs which belonged to the carcass and which were investigated separately, i.e. of skeletal muscle, liver, kidney, intestine, heart etc. (Tables 1 and 3). This indicates that the remaining organs, e.g. pancreas, salivary glands, endocrine glands, lung and bones, are also involved to a considerable extent in the metabolism of DL-[2-^{14}C]dopa.

Subfractionation. One hour after the administration of DL-[2-^{14}C]dopa, the amino acids of brain contained the largest portion of radioactivity in the position of dopa and a smaller portion in that of 3-*O*-methyl-dopa, whereas in liver the latter was predominant.

Conjugation of the amines was almost complete in the blood, liver and intestine, marked (40–60 %) in the heart, skeletal muscle, carcass and skin, and weak in the brain (Table 4). After deduction of the radioactivity due to the presumed blood content of the organs (about 5 %), the amines in the brain are almost entirely unconjugated (Table 4). The absolute concentrations of unconjugated amines

Table 1. *Radioactivity in tissues* 1 *hr. after the subcutaneous injection of* DL-3,4-*dihydroxy*-[2-^{14}C]*phenylalanine in rats*

The values represent averages ±S.E.M. or radioactivity recovered per ml. of blood or per g. of fresh tissue expressed as percentages of the radioactivity injected/g. body wt.

Tissue	No. of expts	Radioactivity recovered (%) Amino acids	Amines	Phenolcarbonic acids	Overall	Amine: amino acid radioactivity ratio
Blood	28	15·1±0·7	3·4±0·1	4·4±0·2	22·9±1·0	0·23
Brain	19	4·4±0·1	0·8±0·1	3·7±0·2	9·0±0·3	0·18
Skeletal muscle*	4	12·2±0·9	2·5±0·4	3·2±0·5	18·4±1·6	0·20
Heart	4	14·1±2·0	3·4±0·2	5·9±1·2	23·6±3·4	0·24
Aorta	4	12·0±4·1	3·8±1·5	7·6±4·1	23·4±9·6	0·32
Liver	6	15·0±1·6	44·0±4·5	9·2±1·0	68·2±6·5	2·93
Small intestine†	1	12·6	38·0	8·9	55·3	3·02
Adrenals	2	16·5±2·7	47·7±28·2	2·9±0·2	67·3±30·5	2·89
Kidney	5	56·1±8·6	35·2±5·6	47·1±6·9	138·0±20·0	0·63
Skin‡	1	41·3	4·5	5·0	50·8	0·11
Carcass§	4	13·7±1·8	7·3±1·5	4·2±0·8	25·2±3·7	0·53

* Upper part of hind legs.
† Duodenum, jejunum and caecum.
‡ Skin including adherent, practically fat-free, subcutaneous connective tissue; hairs were previously removed by an electric razor.
§ Excluding head, paws, tail, skin, the major part of blood, the contents of the intestine and urinary bladder.

Table 2. *Tissue:blood ratio of radioactivity* 1 *hr. after the subcutaneous injection of* DL-3,4-*dihydroxy*[2-^{14}C]*phenylalanine in rats*

The values represent averages ±S.E.M. of the radioactivity per g. of fresh tissue/radioactivity/ml. of blood.

Tissue	No. of expts	Tissue: blood radioactivity ratio Amino acids	Amines	Phenolcarbonic acids	Overall
Brain	19	0·31±0·02	0·24±0·02	0·89±0·05	0·41±0·02
Skeletal muscle*	4	0·61±0·05	0·69±0·13	0·64±0·09	0·68±0·08
Heart	4	0·75±0·05	0·96±0·07	1·40±0·17	0·89±0·07
Aorta	4	0·61±0·21	1·08±0·34	1·76±0·90	0·85±0·33
Liver	6	0·84±0·05	14·36±1·45	2·10±0·16	2·70±0·16
Small intestine*	1	0·87	8·13	2·73	2·54
Adrenals	2	1·11±0·22	12·56±5·79	0·83±0·01	2·31±0·66
Kidney	5	3·50±0·29	10·90±1·70	10·90±0·70	5·89±0·44
Skin*	1	2·73	1·33	1·15	2·22
Carcass*	4	1·14±0·22	2·10±0·30	1·13±0·15	1·29±0·19

* See Table 1.

were very low in the blood, moderate in the brain and liver, and high in the skeletal muscle, carcass, skin, heart and intestine (Table 4). According to differential hydrolysis, the amine conjugates of liver consisted of 23 % of sulphates (hydrolysed by 0·1 N-hydrochloric acid at 100° for 2 hr. under nitrogen) and of 76 % of glucuronates (hydrolysed by β-glucuronidase at pH 5·0 at 37·0° for 3 hr.). After separation of the total amines of the liver by paper chromatography, about half of the radioactivity was present in the position of 3-hydroxytyramine and its methyl ether, but practically none in the position of norepinephrine (Table 5). The conjugates of glucuronic acid showed the same

Table 3. *Radioactivity per whole organ 1 hr. after the subcutaneous injection of* DL-3,4-*dihydroxy-*[2-^{14}C]*phenylalanine in rats*

The values represent averages ±S.E.M. of radioactivity recovered expressed as percentages of the total radioactivity injected.

Tissue	No. of expts	Radioactivity recovered (%)				
		Amino acids	Amines	Phenolcarbonic acids	Overall: Uncorrected	Overall: Corrected*
Blood†	28	0·76±0·04	0·17±0·01	0·22±0·01	1·15±0·05	1·15
Brain	23	0·06±0·002	0·01±0·003	0·06±0·002	0·11±0·004	0·13
Skeletal muscle‡	5	2·99±0·19	0·60±0·08	0·76±0·13	4·45±0·32	5·70
Heart	6	0·01±0·001	0·04±0·01	0·02±0·003	0·067±0·006	0·067
Aorta	4	0·002±0·001	0·007±0·003	0·003±0·001	0·015±0·005	0·015
Liver	6	0·51±0·06	1·42±0·16	0·31±0·03	2·32±0·22	2·68
Small intestine§	1	0·53	1·41	0·37	2·31	2·68
Adrenals	3	0·006±0·001	0·012±0·005	0·001±0·001	0·02±0·005	0·02
Kidney	6	0·41±0·07	0·29±0·04	0·73±0·05	1·15±0·16	1·25
Skin§	1	5·26	0·59	0·64	6·50	7·65
Carcass§	5	8·56±0·94	4·15±0·69	2·57±0·36	15·26±1·70	17·90
Expiration ($^{14}CO_2$)‖	1	—	—	—	0·42	0·42
Urine¶	10	9·70±0·6	23·90±0·7	11·10±0·4	44·70±1·60**	44·70
Faeces††	1	0·32	0·63	0·56	1·51	1·51
Sum‡‡		24·7	29·5	15·2	69·7**	73·5**

* Corrected for incomplete tissue extraction (see the Experimental section).
† Assuming a total blood content of 5 % of body wt.
‡ 27 % of body wt. according to three gravimetric measurements.
§ See Table 1.
‖ Collected in ethanolamine.
¶ Excreted urine plus content of the urinary bladder (probably incomplete recovery from the urinary tract).
** Values presumably too low because of incomplete collection of urine.
†† Content of the whole intestinal tract which was squeezed out and subsequently washed with water.
‡‡ Total of brain, blood, carcass, skin, urine, faeces and expiration.

Table 4. *Unconjugated and total* ^{14}C-*labelled amines of different rat tissues* 1 *hr. after the subcutaneous injection of* DL-3,4-*dihydroxy*[2-^{14}C]*phenylalanine*

The values are means ±S.E.M. of the radioactivity recovered per ml. of blood or per g. of fresh tissue expressed as percentages of the radioactivity injected/g. body wt. The values for unconjugated and total ^{14}C-labelled amines were obtained in samples of $HClO_4$ extracts without and with incubation with glusulase.

Tissue	No. of expts	Radioactivity recovered in ^{14}C-labelled monoamines (%)		Unconjugated amines % of total	
		Unconjugated (uncorrected)	Total (uncorrected)	Uncorrected	Corrected*
Blood	6	0·10±0·03	3·43±0·26	3	—
Brain	5	0·46±0·03	0·58±0·02	79	100
Skeletal muscle†	2	1·29±0·57	2·79±0·38	46	49
Heart	1	3·07	4·66	66	68
Liver	2	0·69±0·45	35·00±10·7	2	2
Small intestine†	1	3·44	37·40	9	9
Skin†	1	2·75	4·56	60	62
Carcass†	3	1·74±0·53	4·48±2·64	39	40

* Corrected for blood content of the organs (about 5 %).
† See Table 1.

Table 5. *Composition of total amines in various organs*

Chromatographic separation of amines was carried out after hydrolysis with glusulase. The values represent percentages of the total amines. Unidentified compounds mainly located at R_F 0·61 accounted for the remaining radioactivity.

		Position of radioactivity			
		Norepinephrine	Dopamine	Normetanephrine	3-*O*-Methyl-dopamine
		Composition of amines (% of total)			
Tissue	No. of expts				
Blood	16	2 ± 1	60 ± 3	—	31 ± 2
Brain	16	24 ± 2	32 ± 2	13 ± 1	12 ± 1
Skeletal muscle*	1	1	71	—	15
Liver	3	2 ± 1	51 ± 3	—	46 ± 2
Kidney	3	3 ± 1	69 ± 4	—	23 ± 2
Urine	4	3 ± 2	86 ± 6	—	10 ± 3
Heart	1	32	37 (Dopamine + Normetanephrine)		14

* See Table 1.

distribution pattern as the total amines, whereas conjugation with sulphuric acid was predominant in the 3-hydroxytyramine fraction. Total amines extracted from the blood, brain, skeletal muscle, heart, kidney and urine consisted mainly of 3-hydroxytyramine, to a lesser extent of 3-methoxytyramine, and, in the brain and heart, also of [14C]-norepinephrine (Table 5).

Preliminary separation of the phenolcarbonic acid fraction of the blood, brain, kidney, liver and urine showed that at least 50 % of the radioactivity was present in the position of 4-hydroxy 3-methoxyphenylacetic acid and 10–30 % in the position of 3,4-dihydroxyphenylacetic acid.

DISCUSSION

In the present experiments with DL-[2-14C]dopa, the radioactivity of the fraction of amino acids in most tissues is probably mainly due to L-dopa. Thus it appears that the D-form of aromatic amino acids enters the tissue less than the L-form (Guroff & Udenfriend, 1962) and is probably excreted in the urine to a larger extent (Gerritsen *et al.* 1961; Pellerin & D'Iorio, 1955; Shaw *et al.* 1957; Sourkes, 1961). The brain does not take up D-dopa in significant amounts *in vitro* (Yoshida, Kaniike & Namba, 1963) or *in vivo* (B. Falck, personal communication). The radioactivity of the amine fraction can only result from decarboxylation of L-dopa, since D-dopa is not degraded by decarboxylase (Blaschko, 1942; Holtz, Heise & Lüdtke, 1939; Lovenberg, Weissbach & Udenfriend, 1962). Although unconjugated amines may be taken up by various organs, especially heart and sympathetic axons (Axelrod, Weil-Malherbe & Tomchick, 1959; Whitby, Axelrod & Weil-Malherbe, 1961; Wolfe, Potter, Richardson & Axelrod, 1962), the endogenous amines appear to be mainly formed *in situ* at least in the heart (Kopin & Gordon, 1963*a*). In any case, in the present experiments the low blood concentration of unconjugated amines (practically no norepinephrine, 10 μg. of 3-hydroxytyramine/l.) presumably did not allow any significant amine uptake by tissues. The phenolcarbonic acids and phenol alcohols may be formed by oxidation of the amines (for reviews see Carlsson & Hillarp, 1962; Daly & Witkop, 1963; Goldstein & Gerber, 1963; Pletscher, Gey & Zeller, 1960), by transamination of L-dopa (Cammarata & Cohen, 1950; Canellakis & Cohen, 1956; Haavaldsen, 1962; Pellerin & D'Iorio, 1955; Pogrund, Drell & Clark, 1961; Shaw *et al.* 1957) and possibly by oxidation of D-dopa (D-amino acid oxidase) (Meister & Wellner, 1963). A small portion of 3,4-dihydroxyphenylpyruvic acid derived from D-[2-14C]dopa is probably transformed into L-dopa by transamination (Holtz & Credner, 1944; Pogrund *et al.* 1961). It is unlikely that most metabolic end products, such as catecholamine conjugates or phenolcarbonic acids, are taken up to a considerable extent by extrarenal tissues, since these metabolites are rapidly excreted into the urine (Pellerin & D'Iorio, 1956/57; Shaw *et al.* 1957). This is confirmed by the present results that the kidney is the only organ which shows an extensive accumulation of phenolcarbonic acids (Tables 1 and 2). Further, no marked penetration of 3,4-dihydroxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid into the brain, heart and other extrarenal organs could be demonstrated (Carlsson & Hillarp, 1962; Goldstein & Gerber, 1963; A. Pletscher & K. F. Gey, unpublished work), whereas phenol alcohols might be able to enter the brain (Goldstein & Gerber, 1963). In the rat, however, dopa and dopamine are transformed to a lesser degree into phenol alcohols than into phenolcarbonic acids (Goldstein & Gerber, 1963).

In conclusion, the following assumptions can be made:

(*a*) The overall radioactivity of a tissue is primarily related to the uptake of L-[2-^{14}C]dopa and partly of D-[2-^{14}C]dopa in extrarenal tissues.

(*b*) The radioactivity of the amine fraction is a qualitative indicator for the rate of uptake and of decarboxylation of L-[2-^{14}C]dopa.

(*c*) The radioactivity of the amine fraction and the amines:amino acids radioactivity ratios (Table 1) depend to a great extent on the decarboxylation of L-[2-^{14}C]dopa.

The kidney can probably not be compared with other organs, since all the three metabolic fractions are excreted in the urine.

From the above considerations, the experimental findings (Tables 1 and 2) indicate the following tissue differences in the uptake and decarboxylation of L-[2-^{14}C]dopa: low capacity (tissue: blood ratio of overall radioactivity markedly below 1) in the brain; medium capacity (tissue: blood ratio about 1) in the skeletal muscle, carcass, heart and aorta; high capacity (ratio markedly above 1) in the intestine, adrenals, liver, kidney and—with respect to the amino acid uptake—also in the skin.

Tissues with a low to medium capacity in uptake and decarboxylation of L-dopa show a high percentage of unconjugated norepinephrine and 3-hydroxytyramine, whereas organs with a high capacity, as far as investigated, contain preferentially conjugated amines and practically no norepinephrine. The adrenals, which are known to store large amounts of unconjugated norepinephrine and epinephrine, are probably an exception. The high amino acid uptake of the skin might be related to special functions of the skin, e.g. melanin formation which was lost in the albino rat.

The uptake of dopa by various tissues seems to be similar to that of other aromatic amino acids, e.g. phenylalanine, tyrosine (Guroff & Udenfriend, 1962) and 5-hydroxytryptophan (Erspamer & Bertaccini, 1962). This suggests a common mechanism that might be as unspecific as the reactions involved in the further metabolism of aromatic amino acids, e.g. hydroxylation (Freedland, Wadzinski & Waisman, 1961; Renson, Goodwin, Weissbach & Udenfriend, 1962), decarboxylation (Lovenberg *et al.* 1962), transamination (Jacoby & LaDu, 1962) and amine oxidation (for reviews see Blaschko, 1963; Pletscher *et al.* 1960). The low radioactivity of the cerebral amino acid fraction demonstrates again the unique property of the brain with respect to amino acid penetration (Guroff & Udenfriend, 1962; Udenfriend & Zaltzman-Nirenberg, 1963), which is probably due to the blood/brain barrier. The uptake of L-dopa, though small, does not seem to be limiting factor for the cerebral amine formation. Thus, in agreement with previous findings in the rabbit (Pletscher & Gey, 1962), but contrary to those in the guinea pig (Udenfriend & Zaltzman-Nirenberg, 1963), the concentration of the amino acid fraction in the brain (probably consisting of a large proportion of L-[2-^{14}C]dopa) remains relatively high for several hours, but the cerebral amines show only a transient increase (Fig. 2). This suggests that either the decarboxylase activity or the availability of dopa for the decarboxylase might have been decreased.

The rate of dopa decarboxylation *in vivo*, as indicated by the present results, is in principle similar to the activity of dopa decarboxylase *in vitro*, being low in the brain, skeletal muscle, heart, aorta and skin, and high in the liver and adrenals (Blaschko, 1942; Davis & Awapara, 1960; Dietrich, 1953; K. F. Gey, unpublished work); Langemann, 1951; Müller & Langemann, 1962; West, 1958). In the intestine *in vivo*, the amine concentrations are relatively high compared with the moderate activity of the decarboxylase *in vitro* (Davis & Awapara, 1960; K. F. Gey, unpublished work); this might be due to some storage of catecholamines *in vivo*. In the kidney, the amine concentrations are rather low in comparison with the high decarboxylase activity *in vitro* (Blaschko, 1942; Davis & Awapara, 1960; Dietrich, 1953), presumably because of further metabolism and excretion, Nevertheless, the ability of the kidney to decarboxylate dopa *in vivo* is evident because of the higher amine: amino acids radioactivity ratio in this organ as compared with blood.

It is known that exogenous catecholamines undergo predominantly 3-*O*-methylation before deamination or conjugation (Axelrod, 1959; Kopin & Gordon, 1963*b*; Whitby *et al.* 1961). The present subfractionation of amino acids, amines and phenolcarbonic acids shows that *O*-methylation is also of importance for the catecholamines derived from exogenous dopa. It further confirms that dopa can also be *O*-methylated (Studnitz, 1961) and that the liver seems to be the most active organ in this respect (Table 5) (Axelrod & Tomchick, 1958).

SUMMARY

1. In rats receiving DL-3,4-dihydroxy[2-^{14}C]-phenylalanine (20 mg./kg.) subcutaneously, the relative total radioactivities of the phenolic amino acid, amine and metabolic-end-product (e.g. phenolcarbonic acids) fractions were measured in various organs and excretion products.

2. After 1 hr., 45 % or more of the injected radioactivity was excreted in the urine, 1·5 % in the faeces and 0·4 % in the respiration; about 8 % remained in the skin, 6 % in the whole skeletal muscle, 3 % each in the intestine and liver, 1 % each in the kidneys and the blood, about 0·1 % in

the brain and heart, and about 0·02 % in the aorta and adrenals.

3. One hour after the injection of DL-dihydroxy-[2-^{14}C]phenylalanine, the overall radioactivity, the radioactivity of the amino acids and the amines/g. of fresh tissue, as well as their tissue: blood radioactivity ratios, were low in the brain, medium in the heart, aorta, skeletal muscle and carcass, and high in the intestine, adrenals, liver and kidney. This order might reflect the relative capacity of tissues to take up and to decarboxylate L-dihydroxy-[2-^{14}C]phenylalanine. The skin contained considerable amounts of the amino acids which seemed to be metabolized only to a moderate degree.

4. According to the time-course established for the brain, the amine formation seems not to depend on the concentration of L-dihydroxy[2-^{14}C]phenylalanine. The brain amines were almost entirely unconjugated and consisted mainly of 3-hydroxytyramine and norepinephrine.

5. The amines, consisting predominantly of 3-hydroxytyramine and to a smaller extent of its *O*-methyl ether, were found to be partially conjugated in the heart, skeletal muscle and skin; in the blood, liver and intestine they were almost entirely conjugated.

6. The radioactivity of the phenolcarbonic acids/g. of fresh tissue and the tissue: blood radioactivity ratio indicated no or only a slight accumulation of these metabolic end products in all extrarenal tissues.

The valuable contribution to the analytical procedure and the skilful technical assistance by Mr E. Bühler is gratefully acknowledged. Thanks are also due to Dr D. E. Schwartz for the combustions of ^{14}C-containing tissue homogenates.

REFERENCES

Axelrod, J. (1959). *Physiol. Rev.* **39**, 751.
Axelrod, J. & Tomchick, R. (1958). *J. biol. Chem.* **238**, 702.
Axelrod, J., Weil-Malherbe, H. & Tomchick, R. (1959). *J. Pharmacol.* **127**, 251.
Bertler, A., Carlsson, A. & Rosengren, E. (1958). *Acta physiol. scand.* **44**, 273.
Blaschko, H. (1942). *J. Physiol.* **101**, 337.
Blaschko, H. (1959). *Symposion on Catecholamines. Pharmacol. Rev.* **11**, 307.
Blaschko, H. (1963). In *The Enzymes*, 2nd ed., vol. 8, p. 337. Ed. by Boyer, P. D., Lardy, H. & Myrbäck, K. New York and London: Academic Press Inc.
Cammarata, P. S. & Cohen, P. P. (1950). *J. biol. Chem.* **187**, 439.
Canellakis, Z. N. & Cohen, P. P. (1956). *J. biol. Chem.* **222**, 63.
Carlsson, A. & Hillarp, N.-A. (1962). *Acta physiol. scand.* **55**, 95.
Daly, J. W. & Witkop, B. (1963). *Angew. Chem.* **75**, 552.
Davis, V. E. & Awapara, J. (1960). *J. biol. Chem.* **235**, 124.
Dietrich, L. S. (1953). *J. biol. Chem.* **204**, 587.
Dresse, A. (1961). *C.R. Soc. Biol., Paris*, **155**, 420.
Erspamer, V. & Bertaccini, G. (1962). *Arch. int. Pharmacodyn.* **137**, 6.
Freedland, R. A., Wadzinski, I. M. & Waisman, H. A. (1961). *Biochem. biophys. Res. Commun.* **5**, 94.
Gamo, T. (1959). *Folia, endocrin. jap.* **35**, 16.
Gerritsen, T., Copps, S. C. & Waisman, H. A. (1961). *Biochim. biophys. Acta*, **53**, 603.
Goldstein, M. & Gerber, H. (1963). *Life Sci.* p. 97.
Goldstein, M., Musacchio, J. & Gerber, H. (1963). *Fed. Proc.* **22**, 389.
Goodall, McC. (1951). *Acta physiol. scand.* **25**, suppl. 85.
Guroff, G. & Udenfriend, S. (1962). *J. biol. Chem.* **237**, 803.
Haavaldsen, R. (1962). *Nature, Lond.*, **196**, 577.
Hall, G., Hillarp, N.-A. & Thieme, G. (1961). *Acta physiol. scand.* **52**, 49.
Hirs, C. H. W., Moore, S. & Stein, W. H. (1953). *J. biol. Chem.* **200**, 493.
Holtz, P. (1959). *Symposion on Catecholamines. Pharmacol. Rev.* **11**, 317.
Holtz, P. & Credner, K. (1944). *Hoppe-Seyl. Z.* **280**, 39.
Holtz, P., Heise, R. & Lüdtke, K. (1939). *Arch. exp. Path. Pharmak.* **191**, 87.
Jacoby, G. A. & LaDu, B. N. (1962). *Biochem. biophys. Res. Commun.* **8**, 352.
Kalberer, F. & Rutschmann, J. (1961). *Helv. chim. acta*, **44**, 1956.
Kirshner, N. (1959). *Symposion on Catecholamines. Pharmacol. Rev.* **11**, 350.
Kopin, I. J. & Gordon, E. K. (1963*a*). *Nature, Lond.*, **199**, 1289.
Kopin, I. J. & Gordon, E. K. (1963*b*). *J. Pharmacol.* **140**, 207.
Langemann, H. (1951). *Brit. J. Pharmacol.* **6**, 318.
Leeper, L. C. (1959). *Symposion on Catecholamines. Pharmacol. Rev.* **11**, 358.
Lovenberg, W., Weissbach, H. & Udenfriend, S. (1962). *J. biol. Chem.* **237**, 89.
Meister, A. & Wellner, D. (1963). In *The Enzymes*, 2nd ed., vol. 7, p. 609. Ed. by Boyer, P. D., Lardy, H. & Myrbäck, K. New York and London: Academic Press Inc.
Montagu, K. A. (1957). *Nature, Lond.*, **180**, 244.
Müller, P. B. & Langemann, H. (1962). *Klin. Wschr.* **40**, 911.
Murphy, G. F. & Sourkes, T. L. (1961). *Arch. Biochem. Biophys.* **93**, 338.
Pellerin, J. & D'Iorio, A. (1955). *Canad. J. Biochem.* **33**, 1055.
Pellerin, J. & D'Iorio, A. (1956/57). *Rev. Canad. Biol.* **15/16**, 371.
Pletscher, A. & Gey, K. F. (1962). *Experientia*, **18**, 512.
Pletscher, A., Gey, K. F. & Zeller, P. (1960). In *Progress in Drug Research*, vol. 2, p. 417. Ed. by Jucker, E. Basel and Stuttgart: Birkhauser.
Pogrund, R. S., Drell, W. & Clark, W. G. (1961). *J. Pharmacol.* **131**, 294.
Renson, J., Goodwin, F., Weissbach, H. & Udenfriend, S. (1962). *Biochem. biophys. Res. Commun.* **6**, 20.
Rosell, S., Sedvall, G. & Ullberg, S. (1963). *Biochem. Pharmacol.* **12**, 265.
Sano, I., Gamo, T., Kakimoto, Y., Taniguchi, K., Takesada, M. & Nishinuma, K. (1959). *Biochim. biophys. Acta*, **32**, 586.
Shaw, K. N. F., McMillan, A. & Armstrong, M. D. (1957). *J. biol. Chem.* **226**, 255.
Sourkes, T. L. (1961). *Rev. Canad. Biol.* **20**, 187.

Sourkes, T. L., Murphy, G. F. & Woodford, V. R., jun. (1960). *J. Nutr.* **72**, 145.
Studnitz, W. von (1961). *Clin. chim. Acta*, **6**, 526.
Udenfriend, S. & Zaltzman-Nirenberg, P. (1963). *Science*, **142**, 394.
Wang, C. H. & Jones, D. E. (1959). *Biochem. biophys. Res. Commun.* **1**, 203.
Wegmann, A., Kako, K. & Chrysohou, A. (1961). *Amer. J. Physiol.* **201**, 673.
West, G. B. (1958). *J. Pharmacol.* **10**, 92T.
Whitby, L. G., Axelrod, J. & Weil-Malherbe, H. (1961). *J. Pharmacol.* **132**, 193.
Wiegand, R. G. & Perry, J. E. (1961). *Biochem. Pharmacol.* **7**, 181.
Wolfe, D. E., Potter, L. T., Richardson, K. C. & Axelrod, J. (1962). *Science*, **238**, 440.
Yoshida, H., Kaniike, K. & Namba, J. (1963). *Nature, Lond.*, **198**, 191.

Biochem. J. (1964), **92**, 308

Changes Associated with the Production of Fatty Livers by White Phosphorus and by Ethanol in the Rat

BY ANNE SEAKINS* AND D. S. ROBINSON*
External Staff of the Medical Research Council, Sir William Dunn School of Pathology, University of Oxford

(*Received* 11 *December* 1963)

Evidence has been obtained in previous studies that the development of a fatty liver, in association with a lowered concentration of plasma lipids, after the administration of ethionine, carbon tetrachloride or puromycin, may be the result of inhibition of the formation of the protein moiety of the plasma lipoproteins in the liver (Harris & Robinson, 1961; Robinson & Harris, 1961; Robinson & Seakins, 1962; Seakins & Robinson, 1963). In the present work the possibility that inhibition of plasma lipoprotein formation could also account for the experimental fatty livers produced in the rat by white phosphorus and by ethanol has been investigated.

METHODS

Female albino rats (Wistar strain) weighing 180–200 g. were used. In the experiments with white phosphorus they were starved for 16 hr. before being given, by stomach tube under light ether anaesthesia, either 1·5 mg. of white phosphorus dissolved in 0·3 ml. of olive oil (test groups) or 0·3 ml. of olive oil alone (control groups). In the experiments with ethanol the rats were fed on their normal diet until either 3 ml. of ethanol–water (1:1, v/v) (test groups) or an isocaloric amount (4 ml.) of a 50% (w/v) solution of glucose in water (control groups) were given, also by stomach tube. All the rats were starved thereafter until they were killed.

Incorporation of DL-[1-^{14}C]*leucine into the proteins of rat liver and plasma* in vivo. At either 2 hr. (white phosphorus groups) or 2·5 or 14 hr. (ethanol groups) after feeding, rats were injected by the tail vein with 20 μc of DL-[1-^{14}C]-leucine (8·3 mc/m-mole) and killed 1·5 hr. (white phosphorus groups) or 2 hr. (ethanol groups) later by exsanguination from the abdominal aorta. The low-density lipoproteins ($d < 1{\cdot}063$), high-density lipoproteins (d 1·063–1·21) and the residue proteins ($d > 1{\cdot}21$) of the plasma were separated by ultracentrifugal techniques and their specific activities, as well as those of the liver proteins, were determined by methods described by Seakins & Robinson (1963).

Incorporation of [^{32}P]*orthophosphate into the phospholipids of rat liver and plasma.* At either 2 hr. (white phosphorus groups) or 16 hr. (ethanol groups) after feeding, rats were injected by the tail vein with 6 μc of [^{32}P]orthophosphate and killed 3 hr. later by exsanguination. The specific activities of the liver and plasma phospholipids were determined as described previously (Seakins & Robinson, 1963) except that no separation of plasma lipoprotein fractions was carried out.

Incorporation of sodium [1-^{14}C]*acetate into the free cholesterol of rat liver and plasma.* Rats were injected by the tail vein with 20 μc of sodium [1-^{14}C]acetate (13 mc/m-mole) at 2 hr. after feeding with white phosphorus in olive oil or olive oil alone and were killed at various intervals thereafter by exsanguination. Samples of plasma and the livers from individual animals were combined into appropriate test and control groups (three animals/group) and the specific activities of the plasma and liver free cholesterol were determined (Seakins & Robinson, 1963).

Concentration of lipids and free amino acids in plasma and liver. The concentrations of esterified fatty acid, cholesterol and phospholipid in liver and plasma were determined on duplicate samples of lipid extracts prepared as described by Harris & Robinson (1961) with the following modifications. In the preparation of the lipid extracts of liver the whole organ was first homogenized in 240 ml. of ethanol and then 80 ml. of ether was added. The resulting mixture was boiled and filtered while hot and the precipitate was washed twice with 50 ml. portions of ethanol–ether (3:1, v/v). Cholesterol determinations in both liver and plasma were carried out by the method of Pearson, Stern & McGavack (1953) on the

* Present address: Cell Metabolism Research Unit, Medical Research Council, Department of Biochemistry, University of Oxford.