2. Moskowitz, M. S., Moskowitz, A. A., Bradford, W. L., Wissler, R. W., *Arch. Path.,* 1956, v61, 245.
3. Block, R. J., Durrum, E. L., Zwieg, G., *Manual of Paper Chromatography and Paper Electrophoresis,* Academic Press, N. Y., 1957.
4. Kerr, L. M., Bauld, W. S., *J. Biochem.,* 1953, v55, 872.
5. Riemenschneider, R. W., Luddy, F. E., Morris, S. G., *Am. J. Clin. Nutr.,* 1958, v6, 587.
6. James, A. T., *ibid.,* 1958, v6, 595.
7. Smith, A. L., Hauk, R., Treadwell, C. R., *Am. J Physiol.,* 1958, v193, 39.
8. Kinsell, L. W., Michaels, G. D., Fukayama, G., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 829.
9. Alfin-Slater, R. B., Aftergood, L., Wells, A. F., Deuel, H. J., Jr., *Arch. Biochem. Biophys.,* 1954, v52, 18.
10. Luddy, F. E., Barford, R. A., Riemenschneider, R. W., *J. Biol. Chem.,* 1958, v232, 843.
11. James, A. T., Lovelock, J. E., Webb, J., Trotter, W. R., *Lancet,* 1957, v1, 705.
12. Klein, P. D., *Arch. Biochem. Biophys.,* 1957, v72, 238.
13. Mead, J. F., *J. Biol. Chem.,* 1957, v227, 1025.
14. Mukherjee, S., Ackaya, K. T., Deuel, H. J., Jr., Alfin-Slater, R. B., *J. Nutr.,* 1958, v65, 469.
15. Hegsted, D. M., Andrus, S. B., Gotsis, A., Portman, O. W., *ibid.,* 1957, v63, 273.

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Quantitative Determination of C^{14}-Tyrosine Absorption in Melanated and Non-Melanated Mouse Tissues.* (25154)

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Historically the study of tyrosine metabolism is coincident with the study of genetically controlled alcaptonuria, a condition characterized by excretion of a tyrosine derivative, homogentisic acid(1). Many investigations as reviewed by Knox(2), and Meister(3), have been carried out on pathways of tyrosine metabolism. More recently, radioactively labelled tyrosine has been employed, with excellent results, in the study of the tyrosine metabolism mechanism(4,5,6). The role of tyrosine in melanosarcoma formation has also received considerable attention(7,8), and in addition the selective absorption of this tagged amino acid into various tissue proteins has been established(9). It is our purpose to study selective absorption of C^{14}-labelled tyrosine by both melanotic and non-melanotic tissues of the mouse by means of quantitative uptake determinations. S91 melanoma tissue is also included in the analyses. Since the method utilized provides a uniform assay procedure and geometry, it is felt that many technical quantitative problems are eliminated; *e.g.*, variation in self-absorption between dissimilar tissue samples.

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Materials and methods. A. The mice used were of the dba/1 Jackson strain subsequently inoculated with Cloudman S91 melanoma. All animals were of approximately the same age and weight (6 mo., 15 g). B. *Radioisotope administration.* Five microcuries of dl-tyrosine-2-C^{14} (Tracerlab, Inc.) were prepared and administered according to standard procedure(7) to each animal. 5 weeks after tumor inoculation. Twelve animals were used during the investigation. C. *Tissue preparation.* Each animal was sacrificed 7 days after radioisotope injection with immediate removal of melanoma, liver, kidney, brain, adrenal, thyroid, and combined skin and hair tissue samples. Each sample was placed in a small pyrex tube, quick frozen by immersion into acetone-dry ice mixture, and stored at 0-4°C until time of analysis. D. *Tissue analysis.* To eliminate variabilities encountered by use of wet weights as measurement of tissue sample and to put all tissues used on a comparable and accurate assay basis, a modification of the Van Slyke-Folch(10,11) tissue combustion procedure for determination of tissue carbon content was employed. The manometrically determined quantity of CO_2 produced, was introduced into barium hydroxide solution

TABLE I. Selective Absorption of C^{14}-Tyrosine/mg Carbon in Melanated and Non-melanated Mouse Tissues.

			Mean (X) values			
			I	II	III	IV
Tissue	No. of animals	No. of samples	mg carbon ($PCO_2 \times F^*$)	Activity ($N_s - N_b$)	$\left(\frac{\text{II}}{\text{I} \times 200\dagger}\right)$ $m\mu C$/mg C	$\left(\frac{\text{III} \times 100 \times 1000}{5\ddagger}\right)$ % tyrosine $\times 10^{-3}$ absorbed/mg C
Melanoma	12	24	2.433	223	.4583	9.17
Kidney	"	"	2.913	191	.3278	6.56
Adrenal	"	12	1.015	49	.2413	4.83
Liver	"	24	3.267	135	.2066	4.13
Brain	"	"	2.706	94	.1737	3.47
Thyroid	11	11	.583	13	.1114	2.23
Skin & hair	12	23	3.792	75	.0989	1.98

* Correction factor (1.027) for Van Slyke combustion apparatus used.
† Tyrosine standard: 200 cpm ($N_s - N_b$; sample cpm minus background cpm) = 1 $m\mu C$ activity.
‡ Total tyrosine activity inj./animal = 5 μC.

and precipitated as barium carbonate. By this method, a precipitated, $BaCO_3$ yield, representing the carbon content of small tissue samples (20-30 mg) could be assayed for its radioactivity content. By similar procedure (12), a series of C^{14}-tyrosine micro-combustion standards was prepared, to relate the obtained number of counts/minute in any tissue analysis to the actual fraction of millimicrocuries of C^{14}-tyrosine present (absorbed) within the sample. Due to the infinitesimally thin $BaCO_3$ layer produced from any sample of tissue, it was unnecessary to make self-absorption corrections between samples. Radioassay procedures were carried out by triplicate planchet analyses of dried $BaCO_3$ samples wtih a windowless gas flow proportional counter and pre-set time scaler. After interpolating the counts/min/mg of carbon (as manometrically determined), specific activity was expressed as millimicrocuries (or fraction thereof) of C^{14}-tyrosine/mg carbon of the tissue sample. The "t" distribution test(13) was used to determine statistical significance of the data. A P value of 0.05 was chosen as the level of significance (95% confidence interval).

Results. Selective absorption of C^{14}-tyrosine in tissues assayed is presented on a comparative basis in Table I. The order of tyrosine uptake by the tissues (/mg carbon as measured manometrically) was melanoma, kidney, adrenal, liver, brain, thyroid, and skin and hair. Despite some individual variations noted in composite assay of any single tissue, significant differences in uptake (Table II) exist between all tissues with the exception of comparisons between: *a*) adrenal tissue and kidney, liver, brain, or thyroid; *b*) thyroid and skin and hair; *c*) liver and brain. Lack of significant difference between these tissues may indicate a relatively equal selective absorption of the amino acid. It is important to note that melanoma tissue selectively ab-

TABLE II. Statistical Evaluation of Selective Absorption of C^{14}-Tyrosine in Melanated and Non-melanated Mouse Tissues.

Tissue	Cases		Melanoma	Kidney	Adrenal	Liver	Brain	Thyroid	Skin & hair
						"t" values			
Melanoma	24			4.03	3.38	6.63	7.59	5.84	9.21
Kidney	"		<.05		1.83	5.15	6.97	6.05	3.20
Adrenal	12		"	>.05; <.10		.82	1.59	1.93	3.24
Liver	24		"	<.05	>.05		1.85	3.23	5.97
Brain	"		"	"	"	>.05		2.33	4.69
Thyroid	11		"	"	>.05; <.10	"	<.05		.46
Skin & hair	23		"	"	<.05	"	"	>.05	

"P" values

sorbed a significantly greater amount of tyrosine than did any of the other tissues studied. The high order of activity noted in kidney tissue may be attributed to biological turnover of tyrosine, with the continual elimination of tyrosine's metabolic by-products by way of the excretory system. Although urinary activity was noted at its highest during the first 72 hours after radioisotope injection, it maintained a much more reduced level during remaining course of experiment.

The high level of C^{14}-tyrosine activity observed in melanoma tissue supports preliminary results(7,8), where conclusive quantitative evidence may be lacking and considerable uptake variation was noted between animals. Of all tissues studied, values obtained for adrenal and thyroid tissues showed the greatest variability. Similar high levels of activity have been reported for these tissues(7), but a great margin of error (25%) was indicated. This was attributed to tissue weight error and low counter efficiency. In our investigation, measurements were made on a standardized basis (determination of carbon content in each tissue sample), thus eliminating this difficulty. With this procedure, the primary source of error is the combustion of extraneous fatty tissue adhering to the desired tissue sample. Such error, which is readily eliminated by careful preparation of the tissue sample, yields a high carbon determination with a correspondingly low radioassay activity/mg of carbon.

Work in progress indicates a much lower kidney activity at 5 weeks after radioisotope injection, thus supporting previous results(8) which indicate the biological turnover rate of tyrosine. It is assumed that the present activity value obtained for kidney tissue is not due entirely to incorporation of tyrosine into tissue proteins, but to elimination by excretion of d-tyrosine and intermediary breakdown products of l-tyrosine metabolism.

The application of the carbon combustion method used demonstrates it as highly superior to other methods. Reproducibility of results, as demonstrated in both analyses of tyrosine standards (actual carbon content *vs.* theoretical carbon content) and most tissue samples, make the technic feasible. The radioassay of C^{14} (as $C^{14}O_2$) precipitated as $BaCO_3$ affords a greater reproducibility than previous technics (tissue slice preparation, etc.) where self-absorption of C^{14} by tissues is a major factor. It is suggested that the present procedure can be employed to further elucidate, on an accurate quantitative basis, numerous metabolic relationships other than that of tyrosine metabolism either *in vivo* or *in vitro*.

Summary. Five microcuries of dl-tyrosine-2-C^{14} were administered to S91 melanoma-bearing mice (dba/1 strain) 7 days prior to time of sacrifice. Melanated and non-melanated tissues were quantitatively assayed for selective absorption of labelled tyrosine and/or its derivatives. The order of uptake (/mg tissue carbon as measured manometrically) was highest in melanoma tissue and progressively lower in kidney, adrenal, liver, brain, thyroid, and skin and hair tissues. The combined technic of tissue combustion and precipitation of CO_2 as barium carbonate provides an extremely sensitive and reproducible activity measurement of labelled tyrosine uptake.

1. Wolkow, M., Baumann, E., *Z. für Physiolog. Chem.,* 1891, v15, 228.

2. Knox, W. E., *A Symposium on Amino Acid Metabolism,* Johns Hopkins Press, Baltimore, Md., 1955, pp836-866.

3. Meister, A., *Biochemistry of the Amino Acids,* Academic Press, N. Y., 1957, pp346-358.

4. Schepartz, B., Gurin, S., *J. Biol. Chem.,* 1951, v180, 663.

5. Dische, R., Rittenberg, D., *ibid.,* 1954, v211, 199.

6. Udenfriend, S., Wyngaarden, J. B., *Acta Biochemica and Biophysica,* 1956, v20, 48.

7. Reid, J. C., Jones, H. B., *J. Biol. Chem.,* 1948, v174, 427.

8. Robertson, C. H., Griffin, A. C., Russell, W. O., Currie, I. W., New, E. E., *Tex. Rep. Biol. Med.,* 1955, v13, 688.

9. Winnick, T., Friedberg, F., Greenberg, D. M., *J. Biol. Chem.,* 1948, v173, 189.

10. Van Slyke, D. D., Folch, J., *ibid.,* 1940, v136, 509.

11. Van Slyke, D. D., Plazin, J., Weisiger, J. R., *ibid.,* 1951a, v191, 299.

12. Van Slyke, D. D., Steele, R., Plazin, J., *ibid.,* 1951b, v192, 769.

13. Snedecor, G. W., *Statistical Methods,* Iowa State College Press, Ames, 1956, pp77-88.

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