Table 2. Molecular weights of γ-globulin and its chains at infinite dilution expressed in thousands.

Whole γ-globulin	H	L
	Weight average	
170 ± 2	53.5 ± 4	23.8 ± 1
	Z average	
173 ± 10	53.5 ± 4	24.3 ± 1

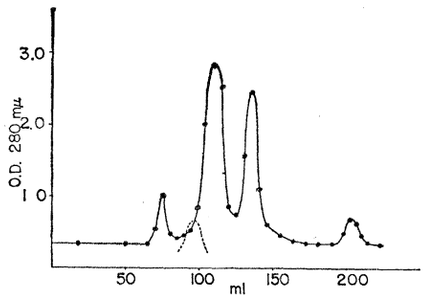

Fig. 1. Representative elution pattern of reduced γ-globulin. In this particular experiment, a 3-ml sample, containing approximately 100 mg of protein, was applied to a column (111 × 1.56 cm) of Sephadex G-200 equilibrated with 5*M* guanidine hydrochloride and 0.1*M* cysteine. The dotted peak represents the position of unreduced 7*S* γ-globulin determined on the same column in a separate experiment. Optical density was measured at 280 mμ in a 1-cm cuvette.

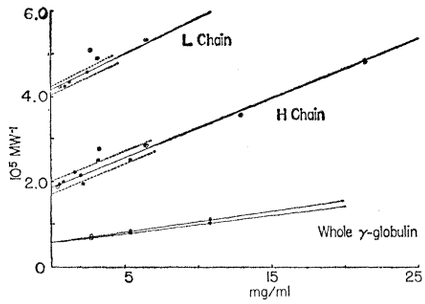

Fig. 2. Concentration dependence of weight-average molecular weights of γ-globulin and its chains. The "dashed" lines represent the deviations given in Table 2.

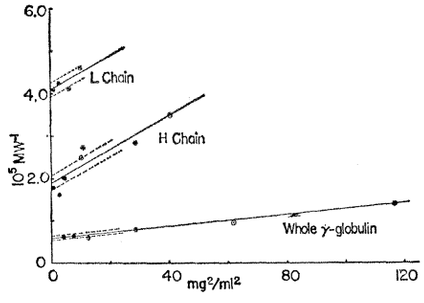

Fig. 3. Concentration dependence of Z-average molecular weights (*11*) of γ-globulin and its chains.

ure 1 shows a typical pattern, and Table 1 shows recoveries and relative amounts of material as determined by absorbancy at 280 mμ. As can be seen in the table, essentially the same pattern is obtained whether sulfhydryl oxidation is inhibited by alkylation, by a reducing agent such as 0.1*M* cysteine, or by a low *p*H. The first peak comes out before native 7*S* γ-globulin and is therefore probably an aggregate. The second peak probably corresponds to the Edelman H (Porter A) chains and the third to the Edelman L (Porter B) chains (*8*). The fourth peak is due to excess alkylating and reducing agents.

Molecular weights were determined in the Spinco model E ultracentrifuge by short-column (3 mm) sedimentation equilibrium in 5*M* guanidine hydrochloride with interference optics (*9*). A partial specific volume of 0.72 was assumed for the whole γ-globulin (*10*) as well as for the chains. The results are summarized in Table 2 and Figs. 2 and 3. Since weight-average and Z-average (*11*) molecular weights agree, the solutions were relatively homogeneous with regard to molecular weight.

Sulfite reduction of porcine γ-globulin is complete even at room temperature in the presence of 5.5*M* guanidine hydrochloride (*12*). Thus, it is likely that under the more rigorous conditions used here, reduction was also complete. The aggregate in the first peak (Fig. 1) is apparently not due to disulfide bonds since a second reduction of this material does not significantly affect its behavior on the column. As the ratio of H chains to the sum of H and L chains (Table 1) is fairly uniform and essentially independent of the variation in the amount of protein in the first peak, it appears probable that the aggregate is composed of both H and L chains in roughly the proportion found in the native molecule.

Thus, assuming equal extinction coefficients for both kinds of chains, it can be seen from the gel-filtration experiments that two-thirds of the mass of the whole molecule (2/3 times 170,000 = approximately 115,000) must be composed of larger chains (molecular weight approximately 55,000 each), and therefore there must be two of them per molecule. By similar reasoning, there are also two smaller chains. These results are consistent with the model proposed by Porter (*4*).

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25 June 1963

Norepinephrine and 3,4-Dihydroxy-Phenethylamine Turnover in Guinea Pig Brain in vivo

Abstract. *By administering C^{14}-labeled tyrosine or H^3-labeled 3,4-dihydroxyphenylalanine to guinea pigs it has been possible to achieve sufficient labeling of the norepinephrine and dopamine in the brain to permit measurement of their turnover rates. The half-life of brain dopamine was about 2.5 hours. The half-life of norepinephrine was about 4 hours, suggesting a rate of synthesis of at least 0.03 to 0.04 μg/g per hr or 2.4 μg/day for the whole guinea pig brain.*

The presence of norepinephrine and its precursor, 3,4-dihydroxyphenethylamine (dopamine), in the brain suggests that these agents play a role in brain function. Such a role is supported by the findings that some centrally acting drugs alter the levels of the catecholamines in the brain (*1*). It was of interest, therefore, to determine the rate of catecholamine formation in the brain in vivo.

It is not possible to make direct measurements in vivo of the rates of norepinephrine synthesis in tissues, but several indirect methods are available. Measurement of the hormone released from the tissue into the venous blood is one possible procedure. Perfusion of radioactive precursors through the isolated organ is another (*2*). Neither of these, however, can be applied readily to the brain. Attempts have been made to estimate the turnover rate of norepinephrine in the heart by admin-

istering radioactive norepinephrine and following its disappearance from the tissue (*3*). Assuming that such a procedure gives correct turnover rates it cannot be applied to the brain since norepinephrine does not penetrate into the central nervous system to an appreciable extent.

Brain, like sympathetic nervous tissue, contains the catalysts required for converting the dietary precursor tyrosine to dopamine and norepinephrine (*4*). It has been possible, therefore, to label the brain catecholamines by administering C^{14}-labeled tyrosine or H^3-labeled 3,4-dihydroxyphenylalanine (dopa) of high specific activity and to estimate the turnover rate from the rate of disappearance of label from the individual catecholamines. One hundred microcuries of tyrosine-3-C^{14} (200 μc/μmole) (*5*) or 200 μc of H^3-labeled dopa (224 μc/μmole) (*6*) were administered intraperitoneally to adult guinea pigs weighing 200 to 250 g. At various time intervals individual animals were killed and decapitated. The brain (about 3 g) was removed immediately and homogenized in 10 ml of 5-percent trichloroacetic acid in a glass, motor-driven homogenizer. The homogenate was centrifuged and catecholamines were isolated by adsorption and elution from alumina (*7*). Aliquots of the alumina eluate were assayed fluorometrically for norepinephrine (*7*) and dopamine (*8*), following which 20 μg of each amine was added as carrier to the remainder of the eluate. The solution was adjusted to *p*H 6 and passed through an IRC-50 (Na^+) column, buffered to *p*H 6 to remove any traces of dopa. Norepinephrine and dopamine were eluted with 1*N* acetic acid and were again chromatographed on a Dowex 50 (H^+) column according to the procedure of Bertler *et al.* (*9*). The purity of the two amines isolated in this manner was ascertained by chromatography on paper, as described previously (*2*). The L-dopa isolated from the IRC-50 effluent was converted to dopamine with *S. faecalis* decarboxylase (*10*) and the latter was further purified by chromatography on a Dowex 50 (H^+) column and assayed fluorometrically as described above. Free tyrosine, which appeared in the alumina effluent, was adsorbed on Dowex 50 (H^+) and eluted with 3*N* ammonia. After evaporation of the ammonia the solution was passed over an IRC-50 (*p*H 6) column. Tyrosine in the effluent was assayed fluorometrically (*11*). Protein-bound tyrosine was isolated as previously described for phenylalanine (*12*). Following fluorometric analysis of an aliquot of each solution, the remainder was transferred to a counting vial containing fluorophor and solvent mixture (*13*), and radioactivity was measured in a liquid scintillation counter. The counting efficiencies, in the solvent used, were 65 to 70 percent for C^{14} and 15 to 20 percent for tritium; background was about 30 to 40 count/min.

Following the administration of C^{14}-labeled tyrosine and H^3-labeled dopa, appreciable amounts of radioactivity appeared in norepinephrine and dopamine. A portion of the data is given in Table 1 as an example of the actual experimental measurements. Following the administration of C^{14}-labeled tyrosine, the specific activities (Fig. 1) reached maximal values within 2 hours and then fell over a period of 16 to 24 hours in a manner characteristic of a first-order decay process. They then leveled off. The specific activity of the precursor, tyrosine, fell extremely rapidly during the first 2 hours, then at a rate not very different from the catecholamines, and finally leveled off. It is apparent that when tyrosine is used as a precursor, turnover studies are limited to the period between 2 and 24 hours, since after 24 hours the protein pool becomes so highly labeled that the specific activity of free tyrosine remains fairly constant, paralleling the comparatively slow turnover of the highly labeled protein pool of tyrosine. From the curves of precursor and products in Fig. 1 it can be seen that one cannot really calculate turnover values for the catecholamines since the precursor, tyrosine, was at all times more highly labeled than the products. Nevertheless, neglecting the error introduced by the presence of labeled tyrosine in the tissue, half-lives ($T_{\frac{1}{2}}$) of at least 3 to 4 hours can be estimated for both norepinephrine and dopamine. Actually these would be expected to be maximal values due to continued resynthesis from the highly labeled tyrosine.

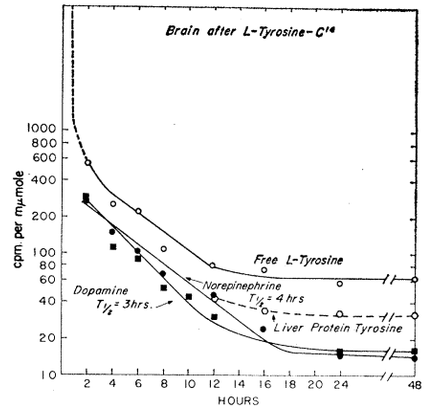

Fig. 1. Decrease in specific activities of norepinephrine, dopamine, and tyrosine in the brain, and of protein-bound tyrosine in the liver, following administration of C^{14}-labeled tyrosine to guinea pigs. Each point represents an individual animal (cpm, count/min).

Table 1. Radioactivity in norepinephrine and dopamine of guinea pig brain following administration of labeled precursors. With the procedures used, approximately 2 mμmole of brain norepinephrine and 3 to 4 mμmole of brain dopamine were present in each counting vial.

Precursor	Time (hr)	Norepinephrine		Dopamine	
		Radioactivity in brain (cpm)	Specific activity (cpm per mμmole)	Radioactivity in brain (cpm)	Specific activity (cpm per mμmole)
L-Tyrosine-C^{14}	2	1054	261	2670	282
	8	270	67	485	52
	16	108	24	271	24
DL-Dopa-H^3	2	2170	842	2745	262
	8	938	288	333	32
	24	202	62	27	3

Table 2. Estimated half-lives ($T_{\frac{1}{2}}$) of norepinephrine and dopamine in guinea pig brain and adrenal gland. The calculations are based on normal concentrations of 0.25 μg/g brain norepinephrine, 0.6 μg/g brain dopamine, and 600 μg/g adrenal epinephrine.

Organ	Norepinephrine			Dopamine		
	$T_{\frac{1}{2}}$ (hr)	μg/g per hr	μg/24 hr	$T_{\frac{1}{2}}$ (hr)	μg/g per hr	μg/24 hr
Brain (3 g)	4	0.033	2.4	2.5	0.11	7.9
Adrenal gland* (.075 g)	300	1.0	1.8			

* The $T_{\frac{1}{2}}$ values for epinephrine and norepinephrine of adrenal gland were taken from the reports of Wyngaarden and Udenfriend (*16*). Those values were obtained on rats and rabbits. However, studies with guinea pigs (*17*) indicate comparably long $T_{\frac{1}{2}}$ values for adrenal epinephrines.

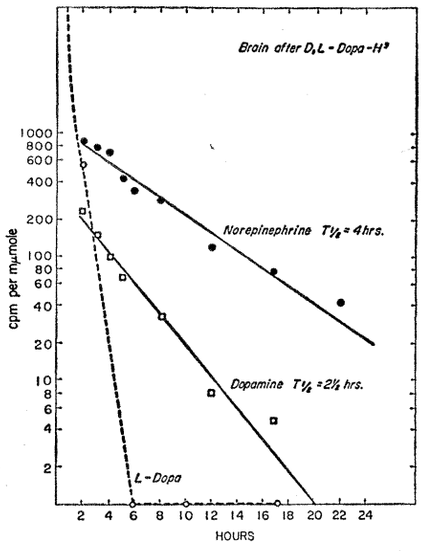

Fig. 2. Decrease in specific activities of norepinephrine, dopamine, and L-dopa in the brain following administration of tritiated DL-dopa to guinea pigs. Each point represents an individual animal.

Tritiated dopa was also found to penetrate into the brain and label the two catecholamines (Table 1 and Fig. 2). However, unlike tyrosine, dopa disappeared from the brain (and blood) extremely rapidly so that decay values for the amines could be obtained without correction for the precursor. It is of interest that approximately the same $T_{\frac{1}{2}}$ values were obtained with dopa as with tyrosine in spite of the relabeling problem associated with the latter precursor. The $T_{\frac{1}{2}}$ values obtained with H^3-labeled dopa are undoubtedly more meaningful.

From the observed $T_{\frac{1}{2}}$ values it is possible to calculate rates of synthesis in vivo. These are shown in Table 2. From the more rapid rate of turnover of dopamine and its higher concentration in the brain, one could conclude that more dopamine is synthesized and metabolized than is utilized for norepinephrine synthesis or that the dopamine pool in the brain is there not merely as a reservoir of norepinephrine precursor, but has a purpose of its own. These findings are not inconsistent with the suggestions by Carlsson *et al.* (*14*) and Sourkes (*15*) concerning a distinct role for dopamine in the central nervous system.

The calculated rates of norepinephrine synthesis in the brain of the normal guinea pig (about 0.03 to 0.04 μg/g per hr) (Table 2) compare to values of 0.05 to 0.10 reported for guinea pig heart when other procedures were used (*2, 3*). Values of this order of magnitude have also been obtained for guinea pig heart and spleen when the same precursor labeling procedure was used (*17*). By contrast, synthesis of epinephrine and norepinephrine in the adrenal gland is much more rapid. However, when values are expressed as micrograms of norepinephrine or epinephrine synthesized in the entire brain or adrenal gland of the guinea pig (Table 2), it is apparent that under basal conditions more of the hormone(s) is made centrally than in the specialized gland for epinephrine synthesis. Comparison with values obtained by similar calculations for other tissues indicates that the catecholamine synthesis in brain represents a major portion of the total catecholamine synthesis in the guinea pig. It should be pointed out that $T_{\frac{1}{2}}$ values are only for catecholamines in storage in the brain. It is conceivable that some norepinephrine or dopamine may be synthesized and metabolized without pooling with the free catecholamines which are present and extractable from the brain. If so, then these $T_{\frac{1}{2}}$ values may be too long; the actual rates of synthesis may be even more rapid than shown in Table 2. Such a mechanism was suggested as an alternative explanation for the extremely long $T_{\frac{1}{2}}$ values observed for adrenal epinephrine and norepinephrine (*18*).

The present findings indicate that norepinephrine synthesis is a rapid process in the central nervous system and further support presently held concepts concerning a neurohumoral function in the brain (*19*).

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Deoxycytidine in Urine of Humans after Whole-Body Irradiation

Abstract. *Increased levels of deoxycytidine were found in the urine of two humans during the first 24 hours after irradiation. This substance presumably was produced by the breakdown of DNA following irradiation, or resulted from interference with the synthesis of DNA.*

Although changes in composition of nucleotides, nucleosides, purine and pyrimidine substances have been reported in tissues and organs in several species (*1*) following irradiation of the whole body, similar changes in these substances have not hitherto been reported in humans. Increased excretion of certain amino acids in the urine for 1 to 6 days after whole body irradiation in nuclear accidents has been reported (*2*); however, we have been unable to confirm that there are any significant differences between the urinary amino acids excreted by cancer patients before or after therapeutic irradiation with cobalt-60 gamma rays, when given in doses of 50 to 200 rad over the whole body (*3*).

Parizek *et al.* (*4*) reported the excretion of deoxycytidine in the urine of rats after irradiation with doses ranging from 10 to 600 r, in amounts proportional to the radiation dosage. Zhulanova and Romantsev confirmed the excretion of deoxycytidine in the urine of rats exposed to high doses (390 to 900 r) (*5*). Later, Kosyakov *et al.* (*6*) reported increased excretion of deoxyribosides by irradiated rats (400 to 800 r) and by dogs (500 r) during the first 24