in Fig. 1. In these two animals, interruptions of flow lasting approximately 8 hours occurred during the 24- to 48-hour post-operative period, when DNA synthesis is at a maximum. These chance observations emphasize the importance of making the competing agent available continuously, as was true for the other seven animals in this group.

Mean grain counts for regenerating liver were calculated for those animals which did, and which did not, receive cold thymidine. Only the 3- to 6-month old mice were analyzed because, in these, baseline labeling was virtually nil. The mean for three animals not receiving cold thymidine was 14.7 radioautographic grains per cell nucleus, with a standard error of 2.1. In four animals which did receive thymidine infusions the mean grain count was 7.4 $\pm$ 0.4. Some of the tritium activity after thymidine treatment may be accounted for by division of the few liver cells labeled prior to hepatectomy. Incomplete suppression of reutilization from extrahepatic sources may also play a role.

That secondary tritium labeling of regenerating liver can be almost completely suppressed by an excess of cold thymidine indicates that the transfer of the tritium label occurs at the nucleoside level. The molecule transferred may be thymidine itself or it may be a related substance which is readily derived from the competing dose of cold thymidine.

In a preliminary experiment concerning the origin of the transferred marker, heavily labeled white cells from mice bearing an A280 tumor, which elicits in the host a granulocytic leukemoid response (*8*), were found to serve as a source of radioactivity for regenerating liver cells. This observation would exclude a specific "trephocyte" function of the lymphocyte which might have been inferred from studies in which transfer of a tritium label was effected by lymphocyte transfusions (see *3* and *4*). Furthermore, it suggests that any tissue in which there is rapid cell turnover can serve as a source of the transferred nucleoside, whether this is derived from an intracellular pool or from the products of DNA catabolism.

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3 July 1963

Polypeptide Chains of Rabbit Gamma Globulin

Abstract. *Rabbit gamma globulin has been extensively reduced, and its polypeptide chains separated by gel filtration on Sephadex G-200 in 5M guanidine hydrochloride. The larger H (or A) chains make up two-thirds of the molecule and have a molecular weight of approximately 55,000 each. The smaller L (or B) chains account for the other one-third and have a molecular weight of approximately 25,000 each. The data are consistent with a model of the gamma globulin molecule that has two H and two L chains.*

Reduction of γ-globulin in the presence of denaturing agents was first reported by Edelman (*1*) who showed that human γ-globulin has polypeptide subunits. He and Poulik (*2*) partially separated and characterized these chains. Franek (*3*) demonstrated that a multichained structure is common to the γ-globulin of several animal species and therefore that analyses of *N*-terminal amino acids are unreliable as indicators of the number of polypeptide subunits per molecule because the number of moles of *N*-terminal amino acid per mole of γ-globulin varies from about 0.5 to 3 for different species (*4*). More recently, on the basis of studies on reduction in the absence of denaturing agents, a four-chain model for the structure of γ-globulin has been proposed (*4, 5*). Conclusive proof for this structure has been lacking, however, because of uncertainties concerning the completeness of reduction, the adequacy of fractionation, and the mass of subunits. In our study, rabbit γ-globulin has been extensively reduced in the presence of a denaturing agent, and the polypeptide chains have been separated. The yields of the two classes of subunits and their molecular weights are reported.

Fraction II of rabbit sera (*6*) was chromatographed on DEAE (diethylaminoethyl) cellulose (*7*) and the eluted protein showed, upon immunoelectrophoresis, a single line characteristic of 7*S* γ-globulin. In all experiments the γ-globulin was then reduced for 1 hour with 0.5*M* β-mercaptoethanol in the presence of a denaturing agent (see Table 1) and 0.1*M* tris buffer, *p*H 8.2. The resulting material was applied to a column of Sephadex G-200 equilibrated with 5*M* guanidine hydrochloride. Fig-

Table 1. Gel filtration of reduced γ-globulin on Sephadex G-200 equilibrated with 5*M* guanidine hydrochloride.

Reduction conditions		S-H "blocking" method*	Recovery (%)	γ-Globulin in peak (%)			
Molarity of guanidine for denaturation	Temperature (°C)			1st	H peak	L peak	$\frac{H}{H+L}$
5	56	Cysteine	95.4	7.0	58.6	34.4	63.0
5	56	HCl	103	8.1	61.5	30.4	66.9
5	56	ICH_2CONH_2	80	9.6	61.0	29.4	67.5
5	56	ICH_2CONH_2	79.3	8.3	63.8	27.9	69.7
6	40	HCl	87.9	2.7	64.8	32.5	66.6
7	25	HCl	95.6	7.3	65.8	26.9	70.9
Sat.	25	HCl	73.7	0	66.0	34.0	66.0
Urea †	45	HCl	95.1	23.0	48.0	29.0	(62.3)
						Average =	67.2

* Three procedures were used to prevent disulfide bonds from forming again after reduction. In some experiments the Sephadex column was equilibrated with 0.1*M* cysteine or 0.1*M* HCl in addition to 5*M* guanidine hydrochloride. For other experiments, the reduced γ-globulin was alkylated by addition of 2 moles of iodoacetamide for each mole of β-mercaptoethanol present during the reduction, and the *p*H was maintained at approximately 8 by addition of 25 percent trimethylamine in water. The resulting solution was then added to the column and the effluent was used for molecular weight determinations. † 11*M* urea was used instead of guanidine.

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

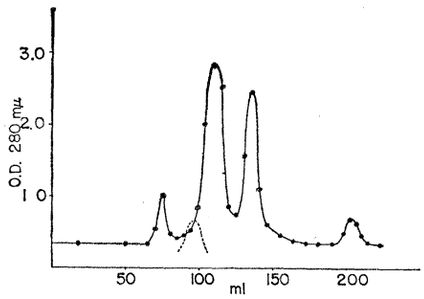

Fig. 1. Representative elution pattern of reduced γ-globulin. In this particular experiment, a 3-ml sample, containing approximately 100 mg of protein, was applied to a column (111 × 1.56 cm) of Sephadex G-200 equilibrated with 5*M* guanidine hydrochloride and 0.1*M* cysteine. The dotted peak represents 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.

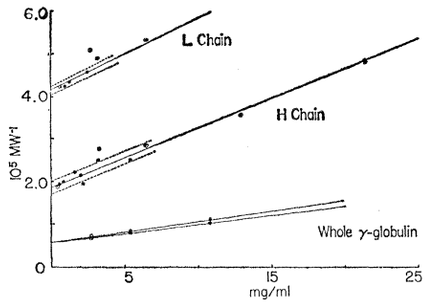

Fig. 2. Concentration dependence of weight-average molecular weights of γ-globulin and its chains. The "dashed" lines represent the deviations given in Table 2.

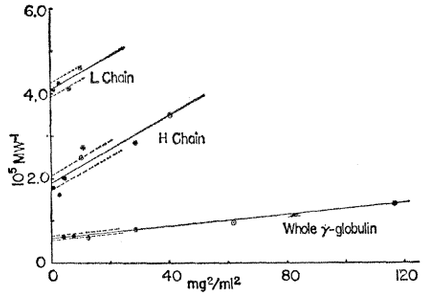

Fig. 3. Concentration dependence of Z-average molecular weights (*11*) of γ-globulin and its chains.

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-