sodium. dl-threonine for instance was found to protect the kidney completely against dl-serine but is without effect on the toxicity of mercuhydrin sodium. These differences are at the present time unexplained.

Summary. Certain amino acids reduce considerably the nephrotoxic action of meralluride sodium in the rat. Among the amino acids examined l(+) arginine monohydrochloride and glycine were found to be most effective. The beneficial influence seen in microscopic sections as well as in the reduced proteinuria following a toxic dose of the mercurial diuretic is attributed to the competitive suppression of tubular reabsorption of the injurious mercury.

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Independent Biosynthesis of Different Hemin Chromoproteins—Cytochrome *c* in Various Tissues.* (18544)

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Since the same prosthetic group, iron protoporphyrin IX (structural type III porphyrin), is present in the different chromoproteins, hemoglobin, myoglobin, cytochrome *c* and catalase, it becomes of fundamental interest and importance to know whether the biosynthesis of the metalloporphyrin (protohemin) is confined to a localized site or whether porphyrin and hemin synthetic ability is a general property of cells of different tissues. Actually, very little information of decisive character is available as to the biosynthesis of chromoproteins other than hemoglobin. Experiments by Beinert *et al.*(1,2) on the incorporation of the radioactive iron isotopes in cytochrome *c* proved merely that if sufficient iron was supplied it would appear also in the prosthetic group of the widely distributed tissue pigment, cytochrome *c*, but left unsettled the question of relative degrees or rates of incorporation in the different chromoproteins, as the labelled iron was furnished in large doses during active growth from birth on, yielding rather similar very high levels of incorporation in all isolated pigment samples. On the other hand, in Theorell's laboratory, it has been shown with radioactive iron that the rate of incorporation of the iron in the hemin of liver catalase is significantly greater than in that of red cell catalase, from which it was deduced that the biosynthesis of the catalases of these respective tissues may be independent.‡ Work in our laboratory on the appearance of "new" cytochrome *c* in liver regenerating after partial hepatectomy(3-5) favored the inference that this chromoprotein was fabricated in tissues *in situ,* but the experiments were indecisive since the possibility of the mobilization of cytochrome *c,* its transfer to regenerating liver from tissues such as skeletal muscle, was not eliminated. A stronger deductive inference of the independent biosynthesis of cytochrome *c* could be drawn from the rapid changes in the cytochrome *c* content of various tissues after thyroidectomy and induced hyperthyroidism(6).

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† With the technical assistance of Anna May Dych, Jean Randall, Charlotte Glausser and Pauline A. Corneille.

‡ Personal communication from Professor Hugo Theorell, Director of the Biochemical Department, Medical Nobel Institute.

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TABLE I. Radioactivity of Cytochrome *c* (*C*) and Hemoglobin (*H*) After Parenteral Administration of Glycine-2-C^{14}.*

Rat	Days after partial hepatectomy	No. of doses of isotope*	Counts/min./mg†				
			Liver *C*	Skeletal muscle *C*	Kidney *C*	Heart *C*	Blood *H*
1 (control)		8	119‡	196	428§		118§
2 (control)		8	715	404			
3	11	8	1039	108	591	47	188
4	6	5	2850	192	474		

* See the text.

† Measurements were made with a windowless counter, and the counts were corrected for self-absorption. The counts per mM (1 iron atom equivalent wt) may be obtained by multiplying the cytochrome *c* counts per mg by 13000 and the hemoglobin counts by 16700.

‡ This result may be low owing to technical difficulties in the preparation of the sample.

§ Pooled sample from rats 1 and 2.

The present report is upon chromoprotein labelling obtained after the administration of glycine-2-C^{14}, and the interpretation of the findings.

Experimental procedure. Adult albino rats of 200 g body weight on a high protein diet (3,4) were used. Liver lobectomy in which 68 per cent of the liver was excised was performed as previously described(4). The glycine-2-C^{14} was administered intraperitoneally in similar amounts to intact controls and to the operated animals in equal divided doses over a period of 5 to 8 days. Each dose was 0.5 ml of aqueous glycine, containing 0.5 mg of the amino acid, with 5 μc of original activity (108,000 counts per minute per μc).

Cytochrome *c* was isolated by the method of Rosenthal and Drabkin(7) and the quantity was determined by our direct micro spectrophotometric technic(7). The isolated material was diluted 50- to 100-fold with highly pure non-radioactive cytochrome *c* (analysis = 0.414% iron; theory = 0.43)§ as carrier, and the mixture dissolved and then resubjected (for purification)(8,9) to the steps of the isolation procedure, including thorough washing with non-radioactive glycine solution to minimize "quasi-incorporation." The cytochrome *c* was finally plated for counting by precipitating it with acetone. The hemoglobin from thoroughly washed red cells was also precipitated with acetone. The precipitate was washed with inactive glycine, and plated from acetone suspension.

Results. Table I is a summary of data on the radioactivity of samples of cytochrome *c* from various tissues and of hemoglobin, after the administration of glycine-2-C^{14}. Attention is directed to the following: (1) The carbon of glycine-2-C^{14} was actively incorporated in the cytochrome *c* of tissues. (2) Appreciably higher activity was obtained in the cytochrome *c* of regenerating liver than in that of the control liver. (3) Muscle cytochrome *c* had a much lower specific activity, and, hence could not be the source of liver cytochrome *c*, unless the unlikely assumption be made that muscle contained more than one species of cytochrome *c*, one of which was highly active (labelled) and preferentially migrated from the muscle. (4) The degree of labelling of the cytochrome *c* of different tissues was different. This could be partially due to the dilution effect of the total cytochrome *c* content, as in the case of skeletal muscle, which contains about 77% of the total cytochrome *c* of the body(3-5), but hardly explains the unusually low incorporation of C^{14} in the cytochrome *c* of heart. This very interesting finding in a tissue of small relative mass, with a high concentration level of cytochrome *c* and high oxidative metabolism(3,5), is unfortunately limited to this isolated instance, and will be subjected to further investigation. It is suggestive of a lower "turn-

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§ Kindly supplied by the Wyeth Institute of Applied Biology (cf. 9).

8. Drabkin, D. L., *J. Biol. Chem.*, 1941, v140, 373.

9. Tint, H., and Reiss, W., *J. Biol. Chem.*, 1950, v182, 385 and 397.

TABLE II. Comparison of C^{14} Incorporation in Liver Cytochrome *c* and Blood Hemoglobin and Provisional "Turnover" Rates.*

Chromoprotein	Total in organ, mg	Total "new" chromoprotein, mg	Total "old" chromoprotein, mg	Mean activity of total chromoprotein counts/min./mg	Calculated activity of "new" chromoprotein counts/min./mg	Calculated "turnover" (provisional) %/day
Liver cytochrome *c*						
Control	1.450†			715		
Regenerating 6 days	1.323‡	.868‡	.455‡	2850	3970§	
Regenerating 11 days	1.323‡	.868‡	.455‡	1039		12.7‖
Blood hemoglobin	3190†	287¶		188	2090	0.8¶

* See the text.

† Based on Crandall and Drabkin(3).

‡ Based on Drabkin(4,5).

§ Total activity of "old" liver cytochrome $c = 715 \times 0.455 = 325$; activity of 0.868 mg of "new" cytochrome $c = (2850 \times 1.323) - 325 = 3445$; activity per mg of "new" cytochrome $c = 3445/0.868 = 3970$.

‖ Mg of unlabelled cytochrome *c* necessary to replace liver cytochrome *c* to reduce count of 2850 to 1039 in 5 days $= ((2850-1039)/2850 \times 1.323 = 0.841$ mg. Rate of "turnover" $= 0.841/5 = 0.168$ mg of liver cytochrome *c* per day, or $(.168 \times 100)/1.323 = 12.7\%$ per day.

¶ Based on life span of approximately 120 days for mammalian erythrocytes. With a "turnover" of 0.8% per day, 9% of the circulating mature red cell population would be labelled during 11 days.

over" of cytochrome *c* in cardiac muscle.

In Table II the radioactivity data are recalculated for liver cytochrome *c* and blood hemoglobin so as to secure a sounder comparison of the relative degrees of isotopic labelling, under our conditions, of the two chromoproteins. The calculations are explained in the footnotes to the table. It may be seen that after adjustment to the highly different dilution spaces of 3190 mg of total hemoglobin and 1.32 mg of liver cytochrome *c*(3-5), the labelling of "new" cytochrome *c* in liver is only 2-fold greater than that of hemoglobin. Since nearly all of the "new" cytochrome *c* of liver is laid down by the sixth day of the regenerative process, and the quantity of the pigment then remains relatively constant(4), a provisional rate of "turnover" of liver cytochrome *c* may be calculated from the values of activity at 6 and 11 days following partial hepatectomy. The "turnover" value calculated from the data is 12.7 per cent per day, which is appreciably greater than the normal daily "turnover" of hemoglobin of approximately 0.8%(10). It is of interest that the "turnover" deduced by this method is of the same magnitude as that previously calculated by us from the rate of appearance of "new" cytochrome during active liver regeneration (4).

Discussion. It has previously been established that glycine is an important intermediary metabolite in the biosynthesis of hemoglobin(11). The present finding of active incorporation of C^{14} from glycine-2-C^{14} into cytochrome *c* suggests that this amino acid probably participates in chromoprotein biosynthesis in general. However, it should be pointed out that the use of a common precursor does not preclude independence of hemin and chromoprotein biosynthesis in the sense of the capability of different tissues of doing the synthetic job. The possibility that muscle cytochrome *c* can migrate to liver and account for the increase in "new" cytochrome *c* during liver regeneration has been excluded by the present findings. Also, the data lend strong support to the view that liver cytochrome *c* is produced *in situ* and independently of the cytochrome *c* of other tissues and hemoglobin. Parenterally injected cytochrome *c*(12) and labelled cytochrome *c* containing radioactive iron(2) are not incorporated in tissues.

The present experiments do not serve to

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12. Drabkin, D. L., *J. Biol. Chem.*, 1947, v171, 409.

exclude the possibility that liver cytochrome *c* may move to other tissues, nor do they exclude the possibility for movement from tissue to tissue of mobilizable smaller molecule precursors.

The "turnover" rate of liver cytochrome *c* is regarded as provisional, since at present we possess no information on the glycine pool in localized areas of the body. Degradation of the labelled chromoproteins, now in progress, will reveal whether the ratio of C^{14} incorporation in hemin and in the protein moiety is different for the cytochrome *c* of different tissues and for hemoglobin. It is also desirable to know whether the metabolism of cytochrome *c* in tissues is dynamic, or "static" as in the case of hemoglobin in mature red cells.

Summary. The participation of glycine in the biosynthesis of cytochrome *c* has been demonstrated. Under our conditions, the incorporation of C^{14} from glycine-2-C^{14} was of relatively very low order in the cytochrome *c* of heart and very high order in liver tissue which had undergone active regeneration. The data support the conclusion that in regenerating liver cytochrome *c* is fabricated *in situ* and not derived from other tissues. A rate of "turnover" for liver cytochrome *c* has been provisionally deduced, which is appreciably higher than the normal rate of hemoglobin "turnover." It is tentatively proposed that chromoprotein biosynthesis is a general property of living, aerobic cells.

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Liver Injury Following Administration of α- and β-Longilobine.* (18545)

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The *Senecio* species first attracted attention when they were found to be responsible for the death of cattle and horses in Canada, South Africa, New Zealand, and the United States. Cushny(2) described the effects of 2 alkaloids, senecifolin and senecifolidine, both isolated from *Senecio latifolius,* in dogs, cats and rats. Since that time some 40 alkaloids of *Senecio* have been studied. One of these is longilobine which occurs in *Senecio longilobus,* a plant indigenous to western Texas, and responsible for great cattle losses. Clawson(3) showed *Senecio longilobus* to be one of the more poisonous of the American species of *Senecio.* Horses, sheep and cattle succumbing to a diet of this plant were found to suffer from extensive liver damage. Longilobine was isolated by Manske(4). Adams and Govindachari(5) employing a chromatographic procedure were able to separate longilobine into two components, α- and β-longilobine. Chemical investigation by Warren and his associates(6) proved that β-longilobine is identical in structure with retrorsine.

Previous studies in this laboratory(7-9) showed that Manske's longilobine and retrorsine caused liver damage in white mice follow-

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