Incorporation of Glycine Into Protein Fractions of Nuclei of Liver and Hepatoma.* (23384)

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For comparison of tumor cells with corresponding normal cells it seems particularly pertinent to have information concerning content and metabolic activities of various protein fractions of the nuclei of these cells. Notable progress in fractionation of proteins of the nuclei of normal cells has been summarized by Allfrey *et al.*(1). Daly *et al.*(2) and Smellie *et al.*(3) have determined the relative incorporation of labelled glycine into several protein fractions of the nuclei of liver cells, and Allfrey *et al.*(4) have made a similar study of protein fractions of the nuclei of calf thymus cells. In the present study the proteins of the nuclei of normal liver cells of the adult rat are compared from a quantitative standpoint with corresponding fractions of the nuclei of a transplanted liver tumor. These fractions were analyzed for glycine, and the incorporation of glycine-l-C^{14} was determined.

Methods. The tumor used in these experiments originated in the liver of a rat which had been fed p-dimethylaminoazobenzene. In the fourth-transplant generation it was implanted by trocar into the axillary region on both sides of male albino rats (Carworth Farms) maintained on Purina dog chow *ad libitum*. When the tumors had attained a size of approximately 10 g, the rats, which at this time were of about adult size, were given an intraperitoneal injection of glycine-l-C^{14} (14 μc/100 g body weight) in 0.9% saline. Twenty hours thereafter the rats were sacrificed, and the livers and tumors were removed quickly for the isolation of nuclei by the method of Hogeboom *et al.*(5). The total cytoplasmic fractions of the homogenates of liver and tumor were used for isolation of samples of the total cytoplasmic proteins. An outline of the procedure used for fractionation of the proteins of the nuclei is given in Fig. 1 and 2. Details of the various steps in the procedure are presented below by the numbers with which they are designated in the figures. Procedures used in more than one step in the fractionation are described only once in the text, and the numbers are repeated in the figures. The majority of the steps in the fractionation were performed in a cold room. Unless specified otherwise, centrifugations were at a speed of 2,000 r.p.m. with the horizontal yoke No. 269 of an International refrigerated centrifuge, model PR-1. (1) Nuclei isolated from liver or tumor were suspended in 10 ml of cold 0.05 M sodium citrate (pH 7) and were homogenized in a cold

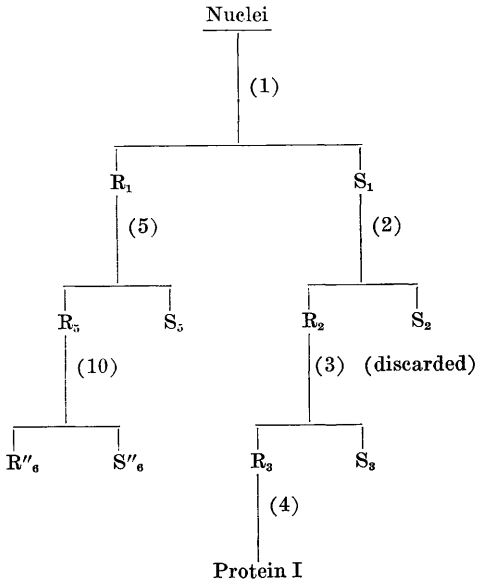

FIG. 1. Outline of procedure used for fractionation of proteins of nuclei. Numbers enclosed in parentheses refer to steps in the fractionation described in the text. S and R refer to supernatant fluid and residue, respectively.

* Supported by grant-in-aid from Am. Cancer Society.

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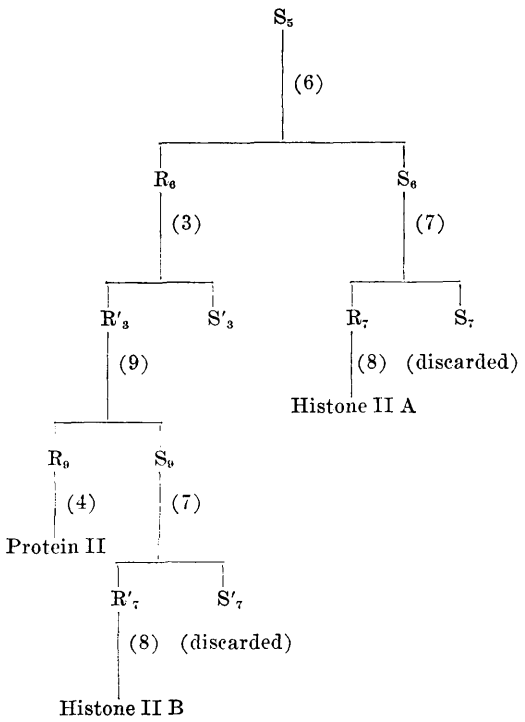

FIG. 2. Outline of procedure used in the further fractionation of S_5. Procedures for further fractionation of R''_6 and S''_6 (Fig. 1) were similar to those for R_6 and S_6, respectively.

micro-cup of a Waring blendor for 1 minute, with repetition of the process until microscopic observation revealed that the majority of the nuclei were fragmented. The homogenate was centrifuged for 30 minutes at 10,000 x G in the multispeed rotor of an International refrigerated centrifuge. The residue was suspended in a second 10 ml portion of cold 0.05 M sodium citrate, and the homogenization and centrifugation were repeated. The remaining residue (R_1) was used in step 5, and the pooled supernatant centrifugates (S_1) were treated according to step 2. (2) Cold 1 N perchloric acid was added with stirring to S_1 to yield a final concentration of 0.1 N. After 30 minutes at 5°, the precipitate was collected by centrifugation, and it was washed 3 times with 5 ml portions of cold 0.1 N $HClO_4$. (3) Residue R_2 was heated for 20 minutes at 90° in 5 ml of 1 N $HClO_4$ in order to hydrolyze and to extract the nucleic acids(6,7). After centrifugation, the residue was washed twice with small portions of 1 N $HClO_4$. The perchloric acid extract and the washings were pooled (S_3) for nucleic acid analyses(6). (4) Protein residue R_3 was washed thrice with absolute alcohol, twice with alcohol-ether (3:1) at 40°, once with ether, and was dried in a vacuum desiccator. (5) R_1 was homogenized in 10 ml of 1 M sodium chloride. The homogenate was agitated in a mechanical rocker for 6 hours at 5°, and was centrifuged at 10,000 x G for 30 minutes. (6) Hydrochloric acid was added, with constant stirring, to S_5 until a final concentration of 0.1 N was attained, and the resulting suspension stirred slowly at 25° for 2 hours. The suspension was centrifuged. (7) S_6 was brought to pH 11 with ammonium hydroxide, and 2 volumes of alcohol were added. The histone fraction R_7 was collected by centrifugation and was washed twice with 75% alcohol. (8) R_7 was purified by extraction with 5 ml of 0.004 N HCl, followed by a second precipitation with ammonium hydroxide and alcohol. The resultant histone was heated with 4 ml of 1 N perchloric acid for 20 minutes at 90°, and the residue was collected by centrifugation and washed twice with 2 ml of 1 N perchloric acid. The perchloric acid extracts and washings were discarded. The residue (histones) was washed 4 times with alcohol, twice with alcohol:ether (3:1) at 40°, once with ether, and was dried in a vacuum desiccator. (9) R'_3 was extracted for 2 hours at room temperature with 5 ml of 0.004 N HCl. The suspension was centrifuged. S_9 was treated according to procedure 7 to yield a small additional quantity of histone which was purified as described in step 8. R_9 was washed and treated by procedure 4. (10) R_5 was extracted by stirring it continuously with 5 ml of 0.1 N HCl at room temperature for 2 hours and centrifuging. The supernatant, S''_6, and the residue, R''_6, were fractionated further by the procedure outlined in Fig. 2 for S_6 and R_6, respectively. There were thus obtained fractions Protein III, Histone III A, Histone III B, and S''_3 which corresponded to fractions (Fig. 2) Protein II, Histone II A, Histone II B, and S'_3, respectively.

Radioactivities of the protein samples were determined in a Tracerlab internal gas flow counter and were corrected for background

TABLE I. Specific Activities of Protein Fractions of Nuclei and Cytoplasm of Liver and of a Transplanted Liver Tumor in Rats 20 Hours after Intraperitoneal Injection of Glycine-1-C^{14}.

Protein fraction	Proteins of liver		Proteins of tumor	
	c.p.m./mg protein	c.p.m./mg prot.-glycine	c.p.m./mg protein	c.p.m./mg prot.-glycine
		× 100		× 100
Total proteins of cytoplasm	1,850	458	940	245
Nuclear fraction I	1,354	286	730	150
Idem II	1,415	367	280	79
" III	1,680	382	820	197
Nuclear histone II	288	46	510	83
Idem III	390	59.7	620	95

and for self-absorption. They are expressed as specific activities in counts per minute per mg of protein, calculated to infinite thinness. Protein samples were analyzed for amino acids by the method of Levy(8) after hydrolysis for 20 hours with 6 N HCl in a sealed tube at 110°. In each case the dinitrophenylglycine was extracted from the paper chromatograms with 5 ml of distilled water, was determined spectrophotometrically, and then was extracted from the aqueous phase with ether. The ether extracts were evaporated upon stainless steel planchets, and the specific activities were determined and were expressed as protein-glycine (Table I).

Results. The gross compositions of the nuclei from the two sources do not differ greatly. The nuclei of the liver contain 30% of protein fraction I, 3% of fraction II, 35% of fraction III and 13% of total histones; corresponding values for the tumor are 25, 4, 32, and 14%. The proteins of fraction I (prior to treatment with perchloric acid) have properties similar to those of globulins. Fraction III corresponds to the "residual chromosomes" of Allfrey *et al.*(1) and to the lipoprotein of Wang *et al.*(9) and Engbring and Laskowski(10). Histone fraction II B was small in comparison with histone fraction II A, and these fractions were combined as histone fraction II for amino acid analyses and for determination of specific activities. This also was the case with histone fractions III B and III A. Glycine contents of the proteins of liver nuclei, as % by weight, were 4.3 for fraction I, 3.5 for fraction II, 4.0 for fraction III, 5.8 for histone II and 6.0 for histone III; corresponding values for protein fractions of tumor nuclei were 4.5, 3.3, 3.9, 5.7, and 6.1. Analyses of extracts S'_3 and S''_3 indicated that they contained deoxyribonucleic acid (or degradation products thereof) in amounts which were approximately equal to the quantities of histone fractions II and III, respectively. The major portion of the ribonucleic acid (or degradation products thereof) of the nuclei was found analytically in extract S_3, but small amounts were also found in S'_3 and S''_3.

Specific activities of the protein fractions of liver and hepatoma are recorded in Table I for one of 3 experiments of this type which were carried out. The results of 2 similar experiments were in general agreement with those of Table I. The major part (90-95%) of the radioactivity of each protein fraction could be accounted for by the radioactivity of the glycine which was isolated from the protein hydrolysates by the method of Levy. However, some radioactivity also was found in the serine, and traces were present in other amino acids. Protein fraction III had the highest specific activity of the various protein fractions of the nuclei of both liver and hepatoma cells, and the activities were only slightly less than those of corresponding total cytoplasmic proteins. Protein fraction I was next in order of specific activities in the tumor, but in liver, fraction II was more active than fraction I. The histones had distinctly lower specific activities than other protein fractions in the nuclei despite the fact that the histones have higher glycine contents than the other protein fractions. It should be pointed out that the histone fractions had much higher specific activities after one precipitation (step 7), but the contaminating radioactivity was removed during the purifica-

tion procedure (step 8). Metabolic heterogeneity of the histones was indicated by the differences in the specific activities of the histone fractions in both liver and hepatoma. Each of the protein fractions of the nuclei of the liver cells had higher specific activities than corresponding protein fractions of the tumor with the notable exception of the histones which were in the reverse relationship. This difference is most readily seen in the ratios of the specific activities of protein-glycine in various protein fractions of the tumor to those of corresponding fractions of liver. These ratios are: total cytoplasmic proteins, 0.54; nuclear fraction I, 0.53; nuclear fraction II, 0.22; nuclear fraction III, 0.52; nuclear histone II, 1.8; nuclear histone III, 1.6. It is not surprising that labelled glycine was incorporated to a greater degree into most of the protein fractions of liver than into corresponding fractions of the hepatoma since the transport of the intraperitoneally injected amino acid to the liver probably would be more favorable than the transport to the subcutaneously transplanted hepatoma. However, the fact that the histones were in the opposite relationship to the other proteins and had higher specific activities in the tumor than in the liver was unexpected, and this observation is of considerable interest. These data suggest that the histones may play a particularly important role in the nuclei of the hepatoma cells. However, further work will be required to determine whether this relationship is characteristic of tumors or whether it is fundamentally characteristic of rapidly dividing cells as contrasted with non-dividing or slowly dividing cells.

Summary. A procedure is described for the separation of the proteins of cell nuclei into several fractions which differ in solubilities and in glycine content. Twenty hours after intraperitoneal injection of glycine-l-C^{14} into adult rats bearing subcutaneously transplanted hepatomas the specific activities of the protein fractions of the nuclei of liver and tumor were in the descending order: residual lipoprotein > globulins > histone fraction III > histone fraction II. Each of the protein fractions of the nuclei of liver cells had higher specific activities than corresponding protein fractions of the hepatoma with the notable exception of the histones which were in the reverse relationship.

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Received May 27, 1957. P.S.E.B.M., 1957, v96.

Methods for Comparing Effects of Various Fats on Fibrinolysis.* (23385)

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Recently, much attention has been directed toward differing biologic effects of various common dietary fats. Ahrens(1,2), Beveridge(3,4), Bronte-Stewart(5), and others have shown in man that fatty meals of substances such as butter (a highly saturated fat

* Supported by grant from the National Heart Institute, N.I.H., U. S. Public Health Service, Bethesda, Md.

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