Serum Glycoprotein Synthesis after Partial Hepatectomy in the Rat

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(Received 27 April 1976)

The incorporation *in vivo* of D-[1-¹⁴C]glucosamine into serum glycoproteins and proteins of liver microsomal fractions shows a decrease in the early stages (24 h) after partial hepatectomy compared with sham-operated animals; 72 h after partial hepatectomy the specific radioactivity of hexosamines bound to liver microsomal fractions reaches the same value as for sham-operated animals.

The effect of partial hepatectomy on liver-protein formation has received much attention [see the review by Bucher & Malt (1971)]. Studies on the rate of synthesis of serum proteins report divergent results. Rosenoer *et al.* (1970) and Murray-Lyon & Muller-Eberhard (1973) found a decrease in albumin synthesis during the early stages of liver regeneration. Becker (1969) observed an increase of albumin and globulin synthesis following hepatectomy. According to Mutschler & Gordon (1966), fibrinogen and transferrin synthesis is increased 24 h after partial hepatectomy. It is known that the secretion of serum glycoproteins by the liver occurs after assembly of carbohydrates which terminate the macromolecule. D-[1-¹⁴C]Glucosamine is an excellent precursor for use in the investigation of synthesis of serum glycoproteins. The amino sugar injected in the rat is rapidly taken up by the liver, bound to the proteins and discharged into the bloodstream (Macbeth *et al.*, 1965).

The experiments described in the present paper were designed to measure the incorporation *in vivo* of [¹⁴C]glucosamine into serum proteins at various times after partial hepatectomy. The specific radioactivity of amino sugars in serum proteins was compared with the specific radioactivity of hexosamines bound to liver microsomal fractions. A rapid method of measuring the specific radioactivity of D-[1-¹⁴C]glucosamine, based on the separation of a volatile chromogen derivative, is described.

Materials and Methods

Male Wistar rats weighing 150–160 g were used. Partial hepatectomy by the technique described by Higgins & Anderson (1931) and sham operation consisting of laparotomy and palpation of the liver were performed under ether anaesthesia. At 24, 48 and 72 h after operation, each animal received a single intraperitoneal injection of 10 μ Ci of D-[1-¹⁴C]glucosamine (The Radiochemical Centre, Amersham, Bucks., U.K.; 58 mCi/mmol) diluted with 5 mg of non-

radioactively labelled D-glucosamine hydrochloride in 2 ml of 0.85% NaCl. At various times after injection, 0.3 ml of blood was taken from the retro-orbital plexus for determining the specific radioactivity of serum glycoprotein hexosamine. To prepare microsomal fractions, animals were killed at various times ranging from 45 min to 24 h after radioisotopic injection and the livers were excised and placed on ice.

Preparation of serum glycoproteins from blood

Each sample of blood collected from the animals was allowed to clot and 0.1 ml of serum was used for each determination. The serum proteins were precipitated by the addition of 20 vol. of 95% (v/v) ethanol and washed three times with 20 vol. of ethanol to remove the free glucosamine. The dried protein precipitates were hydrolysed *in vacuo* in sealed ampoules at 110°C for 4 h in 4.5 M-HCl. The entire hydrolysate was evaporated and dried overnight over P₂O₅ and NaOH pellets in a vacuum desiccator to remove HCl.

Preparation of glycoproteins from liver microsomal fractions

Microsomal fractions were isolated from 1 g of liver by the procedure of Keller & Zamecnik (1956). Microsomal pellets were homogenized with 5 ml of 1% phosphotungstic acid in 0.5 M-HCl. Homogenates were centrifuged, the precipitates extracted twice with the phosphotungstic acid solution, washed once with 5% (w/v) trichloroacetic acid and three times with 10 ml of 95% ethanol. The dried precipitates were hydrolysed and treated as serum-protein precipitates.

Measurement of specific radioactivity of hexosamines

The hydrolysed and dried glycoproteins were boiled in stoppered tubes with 8 ml of pentane-2,4-dione reagent [2% (v/v) in 0.25 M-Na₂CO₃] for 20 min. The solution was transferred into the distillation apparatus described by Cessi & Serafini-Cessi (1963) and 1.2 ml was collected in a calibrated tube. A 0.5 ml

portion of the distilled fraction was placed in a counting vial containing 5 ml of methylCellosolve and 10 ml of scintillation fluid [0.05% 1,4-bis-(5-phenyloxazol-2-yl)benzene and 0.4% 2,5-diphenyloxazole in toluene] and counted for radioactivity in a Packard Tri-Carb liquid-scintillation spectrometer; to another 0.5 ml of the distilled sample, 2 ml of *p*-dimethylaminobenzaldehyde reagent [0.8% (w/v) in ethanol, containing 3.5% (v/v) concentrated HCl] was added, and the E_{548} determined. The distillation apparatus was washed with water and acetone before each distillation. Both radioactivity and E_{548} are a linear function of [^{14}C]glucosamine concentration in the range of 0.05–0.5 μmol .

Results and Discussion

In previous studies (Serafini-Cessi & Cessi, 1970; Serafini-Cessi, 1975) the mechanism of the reaction of amino sugars with pentane-2,4-dione was investigated. The volatile 2-methylpyrrole, which is the main contributor to the colour in the Elson–Morgan reaction, is formed by the reaction of the C-1 aldehyde group of glucosamine with the methenic group of pentane-2,4-dione. By using the method of Cessi & Piliego (1960) for amino sugar determination based on distillation of pyrrole, the radioactivity of D-[1- ^{14}C]glucosamine is linearly transferred to the volatile fraction with a yield of 15%. Because of the specific condensation between amino sugars and pentane-2,4-dione, no neutral sugar or amino acid contributes to the radioactivity in the distilled fraction. In the method used here, the specific radioactivity of hexosamines is easily determined from the same distilled sample, of which one portion is used to measure radioactivity and another to calculate molar concentration after the addition of *p*-dimethylaminobenzaldehyde reagent.

Table 1 shows the hexosamine specific radioactivity of glycoproteins precipitated from the serum of sham-operated and partially hepatectomized animals. A decrease in the incorporation of [^{14}C]glucosamine into glycoproteins appears in regenerating livers. The maximum decrease is 24 h after operation. After 72 h the rates of incorporation are similar in the two sets of animals. The time-course of incorporation in the two groups of animals 72 h after surgery show some differences, however. In regenerating livers the specific radioactivity of protein-bound hexosamine after radioisotopic injection is higher during the first 1.5 h than in sham-operated rats. At later times the specific-radioactivity ratio of partially hepatectomized and sham-operated rats is inverted. The higher specific radioactivity in early periods after radioisotopic injection may be related to the low concentration of hexosamines bound to proteins in the serum of partially hepatectomized rats. I found 5.9 ± 0.27 (5) μmol of hexosamine/ml of serum in sham-operated animals and 4.58 ± 0.25 (5) in rats 72 h after partial hepatectomy. The smaller amount of total serum glycoproteins may favour the rapid increase in specific radioactivity of amino sugars in partially hepatectomized animals. By contrast, the low specific radioactivity at maximum incorporation (3.75 h) and later times may be related to the smaller mass of liver in 72 h regenerating animals compared with control rats.

To obtain more positive information on biosynthesis of serum glycoproteins during liver regeneration, the specific radioactivity of hexosamines bound to liver microsomal fractions was determined (Table 2). In regenerating livers 24 h after operation, the specific radioactivity is halved compared with the sham-operated animals. The values of glucosamine incorporation in partially hepatectomized rats increase with the time from hepatectomy and after

Table 1. Rate of incorporation of D-[1- ^{14}C]glucosamine into hexosamines of serum glycoproteins

Partial hepatectomy and sham operation were performed 24, 48 and 72 h before the injection of D-[1- ^{14}C]glucosamine. At various times after radioisotopic injection, the blood was taken from the retro-orbital plexus of the animals. For experimental details of determination of specific radioactivity, see the Material and Methods section. Each result is the mean value from two rats.

Time after operation (h) ...	Specific radioactivity of hexosamines (c.p.m./ μmol)					
	24		48		72	
	Sham-operated	Partially hepatectomized	Sham-operated	Partially hepatectomized	Sham-operated	Partially hepatectomized
Time after [^{14}C]glucosamine injection (h)						
0.75	277	133	282	225	296	401
1.50	758	375	829	614	827	922
3.75	1466	751	1558	1129	1545	1352
6	1348	692	1298	864	1386	1177
24	489	261	496	316	502	429

Table 2. Rate of incorporation of D-[1-¹⁴C]glucosamine into hexosamines of rat liver microsomal fractions

Partial hepatectomy and sham operation were performed 24, 48 and 72 h before the radioisotopic injection. The animals were killed at the indicated times after the injection of 10 μ Ci of D-[1-¹⁴C]glucosamine. For experimental details, see the text. The results are the mean of two values.

Time after operation (h) ... Time after [¹⁴ C]glucosamine injection (h)	Specific radioactivity of hexosamines (c.p.m./ μ mol)					
	24		48		72	
	Sham-operated	Partially hepatectomized	Sham-operated	Partially hepatectomized	Sham-operated	Partially hepatectomized
0.75	1348	675	1330	1258	1410	1420
1.50	1922	1031	1911	1606	1917	1872
3.75	1554	769	1523	1408	1500	1558
24	432	250	470	360	463	422

72 h the specific radioactivity has reached the same value as in the sham-operated animals. These results demonstrate that a day after partial hepatectomy the synthesis of glycoproteins which are mainly extruded into the bloodstream is significantly decreased. The same change has been reported for albumin by many workers (Mutschler & Gordon, 1966; Lloyd *et al.*, 1975). By contrast, removal of part of the liver leads to an increase in total protein synthesis (Tsukada *et al.*, 1968). According to Braun *et al.* (1962), in the early stages of liver regeneration, precedence is given to the synthesis of cellular proteins and the bio-synthetic activity of secreted proteins is postponed to a later stage. From my evidence, the decrease in glycoprotein synthesis is not a lasting effect, since 3 days after partial hepatectomy the biosynthesis of glycoproteins is restored to normal values. By contrast, Lloyd *et al.* (1975) reported that the synthesis of albumin was still decreased 72 h after partial hepatectomy, whereas Mutschler & Gordon (1966) found the synthesis of fibrinogen and transferrin increased very early after partial hepatectomy. Taken together, all these data indicate that during liver regeneration the synthesis of various plasma proteins proceeds at different and independent rates.

I thank Mr. G. Bellabarba for valuable technical assistance. The financial support of Consiglio Nazionale delle Ricerche, Rome, is acknowledged.

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