α-D-Talose is another pyranose for which both conversion forms seem probable, and a structure investigation of the compound may reveal interesting information about the relative importance of the instability factors.

Unit cell dimensions and space group for α-D-talose are given here together with data for D-*manno*-heptulose and D-*glycero*-β-D-*gulo*-heptose.

Weissenberg and oscillation photographs were taken, using Cu$K\alpha$ radiation, and the densities were measured by the flotation method. The unit cell dimensions given are believed to be accurate to within 0.5 %.

α-D-*Talose*. The crystals are orthorhombic, and the cell dimensions are, a = 8.06 Å, b = 12.17 Å, c = 7.66 Å. Four molecules per unit cell; density, calc. 1.59, found 1.59 g/cm³. The systematic absences are those of the space group $P2_12_12_1$.

D-Manno-*heptulose*. Monoclinic crystals, with cell dimensions, a = 6.60 Å, b = 7.04 Å, c = 9.45 Å, β = 102°. Two molecules per unit cell; density, calc. 1.63, found 1.62 g/cm³. The space group, from systematic absences, is $P2_1$.

D-Glycero-β-D-gulo-*heptose*. Orthorhombic crystals, with cell dimensions, a = 8.59 Å, b = 15.25 Å, c = 6.99 Å. Four molecules per unit cell; density, calc. 1.53, found 1.53 g/cm³. The space group, from systematic absences, is $P2_12_12_1$.

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Further Observations on the Biosynthesis of Polyamines in Regenerating Rat Liver

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In our previous report [1] it was shown that in the chick embryo methionine, ornithine, and putrescine can act as precursors in the biosynthesis of the polyamines spermidine and spermine, whereas no radioactivity was found in the polyamines after administration of ^{14}C-labelled glucose or proline. Subsequently it was demonstrated that in the rat methionine-2-^{14}C and putrescine-1,4-^{14}C are also incorporated.[2] Furthermore, in these studies indirect evidence was obtained that spermidine is a precursor of spermine.

Regenerating rat liver, as a rapidly growing tissue, seemed to be a likely source of further information on the synthesis of polyamines in mammalian tissues. It was shown that in the rat partial hepatectomy causes a rapid and marked stimulation in polyamine synthesis, since a tenfold increase in the specific activity of spermidine compared with the sham-operated controls was observed in the regenerating liver after administration of ^{14}C-methionine.[3,4] In contrast, our preliminary results (unpublished) with labelled putrescine indicated no significant difference between the specific activities of the spermidine isolated from normal liver and that derived from the regenerating organ. This was somewhat unexpected, especially since it has recently been reported by Dykstra and Herbst [5] that the rate of conversion of putrescine-^{3}H to spermidine was almost doubled as little as 2 h after partial hepatectomy. These observations led us to make a more detailed study of the incorporation of putrescine into spermidine during liver regeneration, the possible role of arginine as a precursor of polyamines, and the interconversion between spermidine and spermine.

Material and methods. The animals used were two-month-old female albino rats weighing 135 to 145 g, if not otherwise indicated.

The radioactive material was dissolved in 0.9 % NaCl and administered intraperito-

Table 1. Incorporation of ^{14}C-putrescine into liver spermidine in partially hepatectomized rats. 2 μC (0.23 μmole) of labelled putrescine was administered intraperitoneally 1 h before analysis. The controls were sham-operated 16 h before injection. The values are means for two animals.

Injection time after operation h	Specific activity cpm/μmole
Controls	9 700
0	10 500
2	12 100
6	2 900
10	5 000
16	13 100

neally. Putrescine-1,4-^{14}C dihydrochloride (New England Nuclear Corp., NEN), specific activity 9.1 mC/mmole, was used as such or diluted with unlabelled carrier as indicated in the text. L-Glutamic acid-^{14}C (U) (The Radiochemical Centre, Amersham), spec. act. 6.35 mC/mmole, DL-arginine-5-^{14}C (NEN), spec. act. 3.3 mC/mmole and spermidine-^{14}C trihydrochloride (aminopropyltetramethylene-1,4-^{14}C-diamine, NEN) were used without an added carrier.

Partial hepatectomy was performed under ether anaesthesia by the method of Higgins and Anderson;[6] two-thirds of the liver was removed. Sham-operation consisted of laparotomy only.

Polyamines were analysed by the amido black method after butanol extraction and paper electrophoretic separation.[1] For radioactivity measurements the polyamines were counted directly from the papers in a Packard Tri-Carb liquid scintillation spectrometer.

Results. Table 1 shows the incorporation of putrescine into spermidine at different times during the early period of liver regeneration. There was also some radioactivity in the spermine fractions, but these values are not tabulated. As seen in Table 1, in none of the hepatectomized groups did the specific activity of spermidine significantly exceed that of the controls. It is possible that putrescine is more rapidly eliminated, *e.g.* by binding to newly synthesized ribonucleic acid, in a regenerating tissue than in a normal one. In the next experiment both the amount of putrescine administered and the incorporation time were varied. Again, no great differences were found between the sham-operated and hepatectomized groups (Table 2). Although not conclusive, these results suggest that another source(s) for the four-carbon chain of spermidine and spermine would be stimulated during liver regeneration. Some preliminary observations will be presented here.

No radioactivity was found in the polyamines after administration of 10 μC of uniformly labelled ^{14}C-glutamic acid to a hepatectomized rat. In contrast, after treatment with labelled arginine, the spermidine isolated from both normal and regenerating liver was labelled. Analysis 6 h after injection of 10 μC of 5-^{14}C-DL-arginine at 24 h postoperatively revealed the following specific activities for liver spermidine: sham-operated 1410 cpm, hepatectomized 4070 cpm/μmole. Some activity, although very low, was found in the spermine fraction. These results were consistent with those obtained using a smaller dose and four animals in both groups (Table 3). It can be concluded that arginine is incorporated into liver polyamines, and that its incorporation is

Table 2. Incorporation of radioactivity into liver spermidine in partially hepatectomized and sham-operated rats after administration of 1,4-^{14}C-putrescine. Injection at 20 h after operation.

Incorporation time h	Number of animals	Dose μC	Dose μmoles	Specific activity cpm/μmole sham-op.	Specific activity cpm/μmole hepatect.
0.5	2	4	0.45	22 500	24 000
1	3	4	0.45	19 100	20 700
2	3	4	0.45	28 700	28 300
4	2	4	0.45	44 200	36 100
2	2	4	6.70	19 800	19 800
2	2	4	62.00	7 600	9 600

Table 3. Radioactivity in liver polyamines in sham-operated and partially hepatectomized rats after injection of 2.5 μC of 5-^{14}C-DL-arginine. The animals used in this experiment weighed 110 to 120 g. Injection at 20 h after operation, analysis 4 h later.

Animal No.	Specific activity cpm/μmole			
	Sham-operated		Hepatectomized	
	Spermidine	Spermine	Spermidine	Spermine
1	100	40	1 050	100
2	370	50	1 020	360
3	280	50	1 080	360
4	320	100	1 110	250
Mean	270	60	1 065	270

increased during liver regeneration. Ornithine and putrescine or agmatine may be formed as intermediates.

It was stated previously that we have indirect evidence that spermidine acts as a precursor in spermine synthesis.[1,2] The incorporation of ^{14}C-spermidine (now commercially available) into spermine was shown in regenerating rat liver. In a preliminary experiment after injection of 5 μC of ^{14}C-spermidine at 22 h postoperatively, the radioactivity of liver total spermidine decreased from 14×10^5 cpm at one day after administration to 4.4×10^5 cpm at 5 days and that of liver total spermine simultaneously increased from 1.2×10^5 to 3.3×10^5 cpm. 5 days after injection the specific activity of spermine exceeded that of spermidine.

Studies on *in vitro* synthesis of polyamines as well as their fate in animal tissue are in progress in this laboratory.

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A Polarographic Study of Kinetics and Equilibria of Methyl Green in Aqueous Solutions

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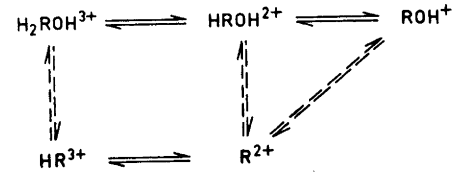
The spectrophotometric investigation of the protolytic equilibria, hydration equilibria, and reaction rates of several basic triarylmethane dyes has been described in a series of papers emanating from this laboratory. Analysis of the dye solutions can also be made polarographically, and conventional polarography has been successfully used for a corresponding study of Methyl Green.

The reactions of Methyl Green in aqueous solutions can be summarized in the following reaction-equilibrium scheme (*cf.* Refs. 1, 2):



R = $[(CH_3)_2NC_6H_4]_2{\cdot}C{\cdot}C_6H_4N(CH_3)_3$.
Whole arrows denote proton transfer reactions proceeding too rapidly to be measured; dashed arrows denote reactions with water or hydroxide ions proceeding at a measurable rate. The equilibria and the reaction rates are described by a set of equilibrium constants and rate constants, which are defined in Refs. 1, 2.