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Turnover Rate of Tissue Proteins in Adult Rats

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The rate of loss of isotope from the mixed proteins of liver, kidney, heart and muscle was studied by injecting L-Methionine-C¹⁴ to adult rats, fed on stock diet. The results indicated that the apparent half-life of proteins of liver and kidney estimated from the slope of the curves were 4.2 days and 5 days, respectively. No significant changes in the activity of heart and muscle protein were observed.

INTRODUCTION

Schoenheimer *et al.* (1939) observed that the amount of labelled amino-acid incorporated into proteins, within a short time after administration of the tagged compound, varied greatly with different tissues. The mixed proteins of some tissues such as intestinal tract, liver and kidney were found to contain large amounts of isotopically labelled amino acid while other tissues such as muscle, skin and brain gave only low values. It was concluded (Schoenheimer and Rittenberg, 1940) that the concentration of isotope found in a particular tissue could be taken as a measure of "relative chemical activity" of the organ proteins.

The turnover rates of various tissue proteins have been measured by using amino acid labelled with different isotopes. Shemin and Rittenberg (1944) by feeding glycine-N¹⁵ found the half life of liver nitrogen and liver glycine to be about 7 days and 5.5 days, respectively. With methionine-S³⁵ either by injection (Friedberg *et al.*, 1948) or feeding (Tarver and Morse, 1948) half lives of liver proteins were 3.7 days and 2.8 days, respectively. Since the half life reflects the rate of catabolism, the proteins with a short half life will be broken down rapidly.

The present experiment was conducted on adult mature rats, to study the rate of loss of radioisotope (L-Methionine-C¹⁴) from the mixed proteins of liver, kidney, heart and muscle.

MATERIAL AND METHODS

Twenty adult sprague-Dawley rats, fed on stock diet (20% protein) were divided randomly into five groups of four rats each. L-Methionine-C¹⁴ (1 μ c/100 gm body weight) was injected intraperitoneally. Each group was killed after 8 hours, 1, 4, 10 and 20 days. The first killing was selected for the reason that the specific activities of tissue proteins reach a maximum at about 1-8 hours, after which they show a gradual decline (Muramatsu *et al.*, 1963).

Liver, kidneys, heart and a piece of gastrocnemius muscle were dissected out, weighed and frozen.

Radioactivity was measured by liquid Scintillation method. Tissues were thawed, minced with scissors and samples of 30-60 mg (liver, kidney, heart) and 100-300 mg (muscle) weighed out and digested in 20 ml glass scintillation vial, fitted with screw tops lined with foil and teflon. Digestion was effected with 0.5-1.0 ml of Nuclear-Chicago Solubilizer (0.6 N base strength in toluene solution) at 50°C overnight or until all the particles had dissolved. The bleaching solution (0.5 ml) of Hansen and Bush (1967) was added to liver samples after complete digestion. The samples were cooled at room temperature for 10 minutes and diluted with toluene and 10 ml of scintillation

solution (Butyl—PBD, (2-(4'-t-Butylphenyl)-5-(4''-biphenyl)-1, 3, 4-oxadiazole) in toluene) to make the final volume of 20 ml in the counting vial. The specific activity (disintegration per minute per mg of tissue) was directly measured in Scintillation spectrometer, standardized internally against the different amounts of unlabelled tissues, treated in identical procedure as mentioned above.

For nitrogen determination Kjeldahl method was used.

RESULTS AND DISCUSSION

The radioactivity (expressed as d.p.m. per mg of tissue or per mg of nitrogen) in liver, kidney, heart and muscle of rats killed at different times after an injection of labelled methionine is shown in Table 1.

Fig. 1 indicates that the level of labelling of liver and kidney was highest (66.7 and 55.2) at 8 hours after the injection, decreased

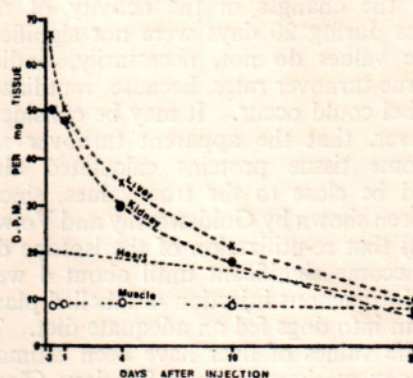


Fig. 1. Radioactivity in liver, kidney and muscle of Rats Fed Stock Diet.

TABLE 1.—Mean radioactivity of tissue proteins.

| | 8 hr. | 1 day | 4 days | 10 days | 20 days |
|-------------------------|-----------|--------|--------|---------|---------|
| No. of rats | 4 | 4 | 4 | 4 | 4 |
| Liver | | | | | |
| *d.p.m. per mg tissue | .. 66.7 | 50.8 | 35.1 | 21.6 | 9.5 |
| d.p.m. per mg. Nitrogen | .. 1961.7 | 1494.1 | 1032.3 | 675.0 | 306.5 |
| Kidney | | | | | |
| d.p.m. per mg tissue | .. 55.2 | 48.3 | 30.1 | 17.9 | 8.3 |
| d.p.m. per mg Nitrogen | .. 1623.5 | 1509.3 | 1204.0 | 526.4 | 267.7 |
| Heart | | | | | |
| d.p.m. per mg tissue | .. 21.5 | 20.5 | 19.6 | 16.6 | 11.0 |
| d.p.m. per mg Nitrogen | .. 632.4 | 621.2 | 612.5 | 518.8 | 333.3 |
| Muscle | | | | | |
| d.p.m. per mg tissue | .. 8.3 | 8.6 | 9.8 | 8.9 | 8.9 |
| d.p.m. per mg Nitrogen | .. 230.0 | 220.5 | 230.0 | 262.0 | 240.0 |

*disintegration per minute.

The rates of decrease of radioactivity (d.p.m. per mg tissue) in each tissue are shown in Fig. 1. The curves were drawn by plotting the average data for the activity of each tissue against the time following injection of L-methionine-C¹⁴.

sharply within the next 16 hours and then fell more slowly.

Heart and muscle tissues appear to show least change in the rate of loss of activity. The average apparent half life of liver and kidney estimated from the slope of the curve

against the 50% initial activity were 4.2 and 5 days, respectively. The half life of heart and muscle could not be calculated since the changes in the activity of these tissues during 20 days were not significant. These values do not, necessarily, indicate the true turnover rates, because reutilization of label could occur. It may be considered, however, that the apparent turnover rates of some tissue proteins calculated above would be close to the true values, since it has been shown by Goldsworthy and Volwiler (1958) that re-utilization of the isotope does not become significant until about 4 weeks after intravenous injection of labelled plasma protein into dogs fed an adequate diet. The half-life values of liver have been estimated by many workers to be 2.8-7 days (Tarver and Morse, 1948; Friedberg *et al.* 1948; Shemin and Rittenberg, 1944; Thompson & Ballou, 1956; Stephen & Waterlow, 1966) which are in agreement with the value measured in this experiment.

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