CHAPTER - VI

P A R T - I.

Study on the Incorporation of C^{14} from Acetate-2-C^{14} and Glucose-U-C^{14} into Proteins and Lipids by Normal and Tumour Cells from Albino Mice.

There is a quantitative difference in normal and malignant tissue regarding growth. Malignancy is associated with abnormal rapid growth and synthesis of lipids and proteins is the main factor in all types of tissue growth. Incorporation of C^{14} in the presence of different labelled substrates into proteins and lipids of normal and malignant human tissue was reported by Banerjee et.al. (1964). In this chapter studies of incorporation of C^{14} from acetate-2-C^{14} and Glucose-U-C^{14} to the proteins and lipids of normal and tumour (SLT) cells in albino mice were undertaken.

Incubation System :

To determine the incorporation of C^{14} from labelled substrates into the proteins and lipids of normal and tumour tissue in mice, incubation was carried in Warburg flasks and materials were added as follows :-

Labelled Compounds (C^{14}) 100 µg/ml	...	0.1 ml.
Magnessium chloride (0.1 M)	...	0.01 ml.
0.1 M Phosphate buffer (pH 7.4)	...	0.3 ml.
Adenosine Triphosphate (0.1 M)	...	0.05 ml.
10% Tissue extract	...	1.00 ml.

0.9% NaCl to make the volume 3 ml.

Labelled acetate-2-C^{14} and glucose-U-C^{14} were obtained from Bhaba Atomic Energy Research Centre, Bombay and A.T.P. from Sigma, New York.

A piece of filter paper soaked in 0.2 ml of 20% KOH was placed in the central well to absorb the liberated CO_2. Incubation was carried out at 37°C, the gas phase being air. After 3 hours of incubation reactions were stopped by tipping in Trichloroacetic acid from the side arm into the main chamber so that the final concentration was 6 per cent. Thus the protein was precipitated in the reaction mixture.

Determination of Radioactivity in Protein after Incubation :

To measure the radioactivity or the incorporation of C^{14} into protein, protein was processed according to Stachiewicz and Quastel (1959) with two additional steps. After stopping reaction with Trichloroacetic acid the contents of flasks were carefully transferred to a hard glass centrifuge tube and centrifuged at 3000 r.p.m. for 10 minutes. The supernatant was discarded and the deposits were washed with 6 ml of

6% T.C.A. and again centrifuged at 3000 r.p.m. for 10 minutes. This procedure was repeated for three times. The deposits were then heated at 90-95°C for 20 minutes. The acid extract was separated by centrifugation and again washed with 6% T.C.A. at room temperature (25°C - 30°C). The deposits were then dissolved in 1 ml of 2 M NaOH and 0.1 ml of 0.1 M sodium acetate solution. The mixture was then kept at 37°C for 45 minutes with occassional mild shaking. To each tube 6 ml of 30% T.C.A. was added and kept for 10 minutes. The solutions were again centrifuged at 3000 r.p.m. for 10 minutes. The precipitates were washed with 6 ml. portions of alcohol-ether (absolute alcohol : diethyl ether 3:1) for 3 times, and lastly the precipitates were washed with ether only.

The alcohol ether mixture and ether pool were kept in a separate container for the study of lipid.

The precipitates were then dissolved in concentrated ammonium hydroxide of reagent quality and transferred carefully to pre-weighed aluminium planchets and dried under infrared lamp. Radioactivity or the incorporation was counted in a windlwless gas-flow counter (Nuclear Chicago) and corrected for background and self absorption.

Determination of Radioactivity in Lipids :

After incubation for 3 hours, protein was precipitated by T.C.A. and processed as described before according to Stachiewicz

and Quastel (1959). After the precipitates were washed with alcohol-diethyl ether mixture (3:1) the latter was pooled in a separate container and evaporated to dryness in a film evaporator at 40°C. The crude lipid was washed to remove other contamination according to Folch et.al. (1957). The pooled lipid extract was filtered through glass wool and kept in a film evaporator. It was then again evaporated to dryness under reduced pressure. The dried material was then dissolved in measured quantity of chloroform methanol mixture (chloroform : methanol = 2 : 1 v/v) and washed according to method of Folch et.al. (1957). To make it free from other contaminations, to the chloroform methanol mixture, water was added and shaken. After 24 hours aqueous layer was removed. This procedure was repeated for three times. The purified lipid extract was then evaporated to dryness in film evaporator and dry lipids were then transferred to pre-weighed aluminium planchets with the help of petroleum ether. The lipids were completely dried and by azotrophic distillation with a few drops of chloroform the last traces of moisture was removed and kept in a vacuum desiccator for over night.

The count for the lipids was taken in a windowless gas-flow counter and corrected for background and self-absorption.

Determination of Protein :

The protein content of the tissue extract was determined colorimetrically by the procedure described by Lowry et.al. (1951). Absorbance at 660 mm was measured in a Klett-Summerson photoelectric colorimeter and compared with values from a standard curve obtained with crystalline bovine serum albumin.

Table - 9.

Incorporation of C^{14} from Different Labelled Substrates into Proteins and Lipids by Normal and Tumour Cells of Mice.

(Expressed as counts per minute per mg. of protein or lipid).

Table shows the mean of 5 different specimens with S.D.±.

Additions	N		M	
	cpm/mg protein	cpm/mg lipid	cpm/mg protein	cpm/mg lipid
Acetate-2-C^{14}	222.5±11.6	540.1±5.7	1050.5±16.0	973.0±8.0
Glucose-U-C^{14}	199.8±13.1	641.6±7.6	1002.2±10.3	1079.8±15.2

N = Normal; M = Malignant tissue.

Results :

Incorporation of labelled carbon by tumour cells from acetate-2-C^{14} and glucose-U-C^{14} to protein was found to be nearly 5 times than the normal ones. Under similar conditions incorporation to lipids was found to be about 1.8 times from acetate and 1.6 times from uniformly labelled glucose.

Results are shown in Table-9.

CHAPTER - VI.

P A R T - II.

Effect of Jawaharene and Mitomycin C on the Synthesis of Protein in Normal and Tumour Cells.

It has been seen ... in the previous part of this chapter that incorporation of C^{14} into proteins and lipids from substrates like Acetate-2-C^{14} and Glucose-U-C^{14} is greater in tumour tissue than that in the normal tissue in mice. In this part of this chapter is has been studied whether Jawaharene and Mitomycin C have any inhibitory effect on the synthesis of protein of normal and tumour cells in mice by incorporation study.

Experiment :-

Tissues were collected and processed as described in part I. of this chapter. Tissue homogenates (10%) of both normal and tumour cells were prepared. Jawaharene solution was prepared freshly just before begining of the experiment. Graded doses of Jawaharene were used (50 μg, 100 μg, 200 μg and 300 ug).

Mitomycin C was available as an amorphous powder in small vial containing 2 mg. It was dissolved in 2 ml of distilled water and used in graded doses of 5 μg, 10 μg, 20 μg and 25 μg in each flask.

Incubation system was same as described in part I of this chapter.

Jawaharene and Mitomycin C were added in graded doses and 0.9% NaCl was added to make the final volume 3 ml. In the control flask no Jawaharene or Mitomycin C was added. A piece of filter paper soaked in 0.2 ml of 20% KOH was placed in the central cup of the flask to absorb the liberated CO_2. Incubation was carried out at $37^{\circ}C$, gas phase being air.

The procedure for measuring the radioactivity is same as described in part I of this chapter.

TABLE - 10

Effect of Mitomycin C on the Incorporation of C^{14} from Alanine-1-C^{14} and Glycine-1-C^{14} to Protein by Normal and Tumour Tissue.

Mitomycin C addition in µg	1-Alanine-1-C^{14}				1-Glycine-1-C^{14}			
	N		M		N		M	
	Counts	% Inhibition	Counts	% Inhibition	Counts	% Inhibition	Counts	% Inhibition
Nil	202.2±9.6	-	1532.6±17.4	-	253.8±5.2	-	1603.2±18.2	-
5	155.2±7.6	23.8	739.9±13.2	51.7	192.4±4.8	24.2	668.4±9.8	58.3
10	133.5±6.9	33.9	592.8±11.3	61.3	148.0±4.6	41.5	504.9±8.9	68.5
20	120.3±7.3	40.4	430.9±9.6	71.8	102.8±3.9	60.3	376.0±6.2	70.3
25	119.8±5.2	40.5	429.9±8.2	71.9	99.6±3.6	61.2	374.8±5.6	70.4

N = Normal tissue; M = Malignant tissue.

The results are expressed as cpm/mg. protein and represent values from 5 different experiments with S.D.±.

TABLE - 11

Effect of Jawaharene on the Incorporation of C^{14} from 1- Alanine-1-C^{14} and 1- Glycine-1-C^{14} to Protein by Normal and Tumour Tissue.

Jawaharene Addition µg	1- Alanine-1-C^{14}				1- Glycine-1-C^{14}			
	N		M		N		M	
	Counts	% Inhibition	Counts	% Inhibition	Counts	% Inhibition	Counts	% Inhibition
Nil	202.2±9.6	-	1532.6±17.4	-	253.8±5.2	-	1603.2±18.2	-
50	182.3±8.9	10.0	1072.4±15.8	30.2	228.2±4.9	10.0	1035.2±11.6	35.3
100	156.2±7.8	24.0	7663.6±16.5	50.2	192.6±4.6	24.2	683.2±8.3	58.3
200	142.4±8.2	30.0	5853.6±15.6	61.8	116.2±5.3	37.0	500.8±5.6	68.7
300	140.6±9.2	31.0	5776.2±14.4	62.3	115.4±5.6	37.8	487.3±5.8	69.0

N = Normal tissue; M = Malignant tissue.

The results are expressed as cpm/mg protein and represent values from 5 different experiments with S.D. ± .

TABLE - 12.

Effect of Mitomycin C on the Incorporation of C^{14} from Acetate-2-C^{14} and Glucose-U-C^{14} to Proteins by Normal and Tumour Tissue.

Addition of Mitomycin C in µg.	Acetate-2-C^{14}				Glucose-U-C^{14}			
	N		M		N		M	
	Counts	% Inhibition	Counts	% Inhibition	Counts	% Inhibition	Counts	% Inhibition
Nil	222.6±13.2	-	1050.5±14.2	-	199.8±6.3	-	1002.4±22.6	-
5	183.8±11.2	16.5	627.9±13.8	40.2	159.7±6.3	20.6	692.6±9.2	31.6
10	168.7±12.2	23.6	436.8±18.2	58.4	152.2±9.6	23.6	451.8±8.2	45.8
20	162.2±11.9	26.8	317.1±4.7	69.8	144.6±9.8	27.6	410.3±6.9	59.2
25	161.7±9.8	27.1	316.2±3.9	69.9	143.8±6.2	27.9	401.2±9.8	60.1

N = Normal tissue; M = Malignant tissue.

The results are expressed as cpm/mg protein and represent values from 5 different experiments with S.D.±.

TABLE - 13.

Effect of Jawaharene on the Incorporation of C^{14} from Acetate-2-C^{14} and Glucose-U-C^{14} to Proteins by Normal and Tumour Tissues.

Addition of Jawaharene in µg.	Acetate-2-C^{14}				Glucose-U-C^{14}			
	N		M		N		M	
	Counts	% Inhibition	Counts	% Inhibition	Counts	% Inhibition	Counts	% Inhibition
Nil	222.6±13.2	-	1050.5±14.2	-	199.8±6.3	-	1002.4±22.6	-
50	206.6±10.6	16.9	729.7±13.6	30.5	177.6±8.4	11.2	882.3±11.9	11.9
100	189.1±10.3	14.8	548.1±11.8	47.8	159.3±9.8	20.2	577.9±6.3	42.3
200	172.2±9.8	22.4	453.6±11.3	56.8	152.2±6.8	23.6	416.8±9.8	58.4
300	170.8±9.9	23.1	443.8±9.6	55.9	151.2±6.9	24.0	416.2±8.9	58.2

N = Normal tissue; M = Malignant tissue.

The results are expressed as cpm/mg protein and represent values from 5 different experiments with S.D.±.

Results :-

Both Jawaharene and Mitomycin C showed profound inhibition of incorporation of labelled carbon from substrates like Acetate-2-C^{14}, Glucose-U-C^{14}, Glycine-1-C^{14} and Alanine-1-C^{14} to protein. Protein metabolism of tumour tissues were found to be much greater than normal tissues. Inhibition of protein turnover by these two antibiotics were many times stronger in tumour tissues than in normal ones, and the inhibition of incorporation was found to be directly proportional to the doses of antibiotics (Tables-10-13).