Metabolism of Glycine-1-^{14}C in the Normal and in the Irradiated Rat

Recent researches conducted by us[1] on the mouse have revealed certain modifications in the utilization in vitro of labelled cystine following irradiation with lethal doses. These investigations have shown an increase in utilization of the substance, which reaches its maximum during the 2–3 days following irradiations and which then tends to decrease although remaining noticeable after six days.

These data partly contradict the general involutive state which the same animals show after irradiation, for which reason it was decided to control the protein metabolism of the liver in vivo instead of in vitro, using another non-sulphurous amino acid so as to establish whether the above-mentioned behaviour was specific for cystine utilization or whether it was the symptom of a particular direction of nitrogen metabolism.

For this purpose we decided to examine the utilization of the glycine-1-^{14}C in the white rat in normal conditions and after irradiation of the whole body with X-rays doses varying.

Methods. For our investigations, 30 white rats of the Wistar strain were used, with weights ranging from 180 to 220 g, all of which came from the same stud and were kept in the same surrounding conditions and under the same dietary regime for the whole duration of the investigations. 10 rats were set aside as control and underwent no treatment of any kind, while the other 20, divided into two lots of 10, each received 500 R and 1000 R respectively in a single dose over the whole body, with the following characteristics: 120 kV, 10 mA, f.d. 30 cm, filter 2 mm Al.

Five days after irradiation, all the animals, including the non-irradiated rats, kept as control, were injected intraperitoneally with 20 μc of glycine-1-^{14}C dissolved in a physiological solution. 1 h after the glycine injection, all the animals were killed, under superficial ether anaesthesia, by taking all blood possible from the abdominal aorta with an heparinized syringe and then washing the animal itself completely with abundant quantities of physiological solution, so as to obtain the organs free of blood.

The blood drawn off was kept for the determinations on plasma, while the washed liver was removed and exactly weighed quantities were used to determine the dry weight and to evaluate the radioactivity of the proteins and the polypeptides. The same determinations were carried out on the plasma according to the methods given in another paper (NOTARIO et al.[2]).

The values obtained through evaluation of the radioactivity of the dried extracts with the Geiger-Müller counter have been calculated in count/min/g of dry liver and in count/min/cm^3 of plasma.

Description of results and conclusions. From an examination of the Table it becomes clear how irradiation has induced in the rat an evident lowering of the hepatic utilization of the labelled glycine. The values of radioactivity of the liver proteins, as well as the values of the polypeptides, are, after irradiation of the whole body, clearly inferior to those appearing in the non-irradiated controls, independently of the size of the X-ray dosage used.

In both cases, in fact, both the average values of the different groups of animals, as well as the single values of determination reveal that the radioactivity of the proteins and the polypeptides is more or less constantly found to be between values that are below the lower limit of oscillations recorded in the control animals.

A very similar pattern is noticeable in the radioactivity of the proteins and polypeptides of the plasma of the same animals. In this case also, radioactivity values of the various fractions after irradiation of the whole body are more or less constantly below the lower limit of normal oscillations.

Interesting data are also obtained from an analysis of the percentage distribution of radioactivity between the two fractions of the protein metabolism examined (proteins and polypeptides). In the liver it is noted that the overall reduction in incorporation of the glycine after irradiation of the whole body is essentially to be attributed to the reduced radioactivity of the polypeptides, while percèntually the radioactivity of the proteins is less reduced. On the other hand, the opposite is the case with the proteins and polypeptides of the plasma, of which a

[1] V. RICOTTI and A. NOTARIO, Minerva nucl. 7, 18 (1963).
[2] A. NOTARIO, V. RICOTTI, and A. ZANETTI, *Regelazione endocrina del metabolismo eritrocitario* (Ed.: VISCONTEA; Collana Haematologica, Pavia 1962).

Absolute values (in count/min/g of dry tissue) and percentage values of distribution of radioactivity among the proteins and polypeptides of the liver and serum

Fractions considered	Controls		500 R		1000 R	
	Absolute values	Val. %	Absolute values	Val. %	Absolute values	Val. %
Liver						
Protein radioactivity	295.8 ± 29	36.4	196.1 ± 71 $P = 0.001$	33.7	214.7 ± 68 $0.01 > P > 0.001$	34.3
Polypeptide radioactivity	515.8 ± 88	63.6	385.6 ± 84 $P < 0.001$	66.3	410.3 ± 113 $0.1 > P > 0.05$	65.7
Serum						
Protein radioactivity	53.1 ± 9.3	72.9	28.1 ± 7.9 $P < 0.001$	65.5	31.6 ± 12.4 $0.01 > P > 0.001$	70.9
Polypeptide radioactivity	19.7 ± 6.9	27.1	14.7 ± 5.4 $0.1 > P > 0.05$	34.5	14.2 ± 5.1 $P = 0.05$	29.1

fall is noticed above all in the radioactivity of the former with a less pronounced percentual reduction in the radioactivity of the latter.

Apart from these variations in the percentage values of the radioactivity distribution of the labelled glycine between the proteins and polypeptides of the liver and the serum respectively, the fundamental datum that remains as the result of these present enquiries is that of a clear slowing down of the intake of the glycine itself in the proteins and polypeptides as a consequence of the massive irradiation of the rat.

These data agree with the alterations of the involutive type that the irradiated animals present, including the loss of weight, while contrasting with what we discovered in relation to the utilization in vitro of the labelled cystine, which induced us to think that the present data obtained by us with glycine correspond more nearly to the direction that the nitrogen balance assumes in the irradiated animal, a direction essentially of the deficiency type. It is moreover possible that the modifications described above for the cystine may be ascribed to an increased request by the tissues of the irradiated animal for sulphur compounds. Further researches conducted with the latter substance in vivo instead of in vitro may confirm or not such a supposition, in favour of which are to be placed the positive results of the therapeutic use of the same either alone or associated with cysteamine in the treatment of radiation disease.

Riassunto. Gli autori hanno esaminato il comportamento dell'utilizzazione della glicina-1-^{14}C da parte del fegato di ratti irradiati e la sua incorporazione nelle proteine e nei polipeptidi del fegato e del siero. I risultati ottenuti hanno messo in evidenza un abbassamento dell'utilizzazione della sostanza, con riduzione, rispetto agli animali di controllo non irradiati, della radioattività delle proteine e rispettivamente dei polipeptidi sia epatici che plasmatici.

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DOPA Decarboxylase Activity in a Transplantable Hamster Melanoma

The urine of patients with generalized melanoma contains large amounts of homovanillic acid[1], a natural metabolite of 3,4-dihydroxyphenylalanine (DOPA)[2], which is actively produced in melanotic tumours[3] and which is believed to be the precursor of melanin[3]. Homovanillic acid may be produced from DOPA via dopamine, and accordingly it was assumed that the increased urinary excretion of homovanillic acid in melanoma patients was due to an increased production of dopamine. This hypothesis prompted an investigation into the dopamine content and the DOPA decarboxylase activity of a melanotic tumour.

For this purpose a transplantable malignant hamster melanoma (type M. Mel. 1 of Fortner, described by ŠALAMON and STORCK[4]) was used[5]. Histologically the tumour showed signs of active melanin formation during the first passages but, coincident with more vigorous growth, the melanin formation decreased and only small amounts of melanotic pigment were revealed by staining according to MASSON and LILLIE[6].

Catechol amines were extracted and isolated by means of an ion exchange procedure[7] and determined fluorimetrically[8]; DOPA decarboxylase was studied by incubating the tumour tissue with C^{14}-labelled DOPA. If not otherwise stated, 10 mg samples of carefully minced tumour tissue were incubated with 3 μg C^{14}-DL-DOPA in 1 ml 0,1 *M* phosphate buffer, pH 7.0, in an atmosphere of nitrogen at 37°C. The DOPA decarboxylase activity was determined by measuring the amount of C^{14}-dopamine produced in 1 h[9,10]. The values are given in mμg dopamine (free base) produced. The activity of monoamine oxidase was determined by means of a method using C^{14}-labelled serotonin as substrate[10,11].

After the fifth passage the dopamine content of the tumour was studied in the following four passages. In the first of these passages significant amounts of dopamine (3.9, 6.7, 7.2 and 7.9 μg/g wet weight) were found in the tumours of the four animals studied. At this stage the tumour was stored for about two months in the deep freeze before renewed transplantation. In all tumour transplants following this treatment dopamine was absent. No explanation of this change of characteristics of the tumour can be offered at present. Neither noradrenaline nor adrenaline could be demonstrated in any experiment before or after deep-freezing the tumour. After administration of DL-DOPA (200 mg/kg i.p.) to tumour-bearing animals, dopamine (mean value 0.7 μg/g wet weight, range 0.2–1.6 μg) could be demonstrated in the tumour tissue 2 h after injection. Similar treatment with L-tyrosine (100 mg/kg) failed to produce any increase in the dopamine content.

The DOPA decarboxylase activity of the tumour tissue was found to be high throughout the experimental period and was unaffected by the deep-freezing. In a consecutive series of determinations on tumours of 16 animals, the

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