

THE INCORPORATION OF PHENYLALANINE- C^{14} INTO THE PROTEINS OF SARCOMA 45 AND OF THE NORMAL ORGANS OF RATS

Pan Tsi-Tsao

Laboratory of Experimental Chemotherapy (Head — Corresponding Member,
Academy of Medical Sciences, USSR, Prof. L. F. Larionov), Institute of Ex-
perimental and Clinical Oncology (Director — Active Member, Academy
of Medical Sciences, USSR, N. N. Blokhin) of the Academy of Medical
Sciences, USSR, Moscow

(Presented by Active Member, AMS, USSR, N. N. Blokhin)

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Notwithstanding the fact that a significant amount of research exists on the study of the incorporation of amino-acids labelled with radioactive isotopes into the proteins of animal organs and tissues, we have failed to find any kind of detailed reports on the distribution of phenylalanine in the organism. Study of the incorporation of this specific aminoacid into the proteins of animal tumors and organs has particular interest in view of the fact that one of the most active tumor-resistant preparations, sarcocystin (p-di (2-chlorethyl) aminophenylalanine), is a chlorethylamine derivative of dl-phenylalanine [2, 7]. In addition, phenylalanine is a component of three peptide-like compounds of p-di-(chlorethyl) aminophenylacetic acid which we have studied [7]; these have a clearly-expressed, tumor-resistant action.

The task of the present research is to study the incorporation of labelled phenylalanine into the total protein fraction of tumors and organs of rats.

METHODS

We carried out the experiments on white rats (weighing 120-160 g.) with subcutaneously-implanted sarcoma 45 [4]. The animals were supplied the usual fodder; after administration of phenylalanine they received no food.

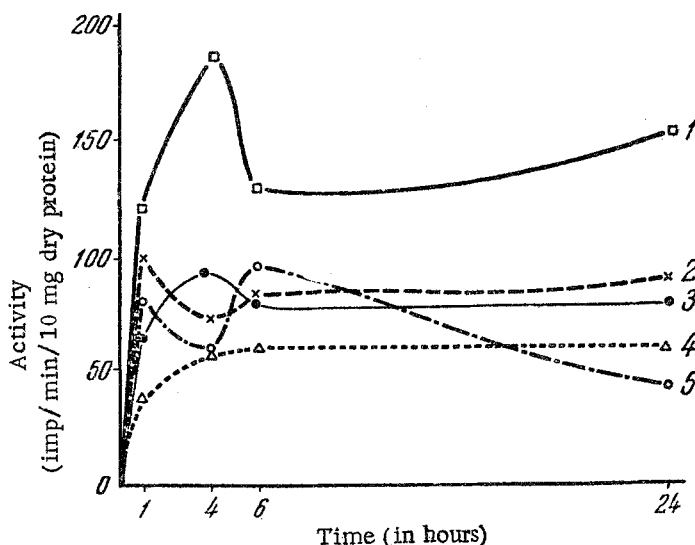
The radioactive phenylalanine was labelled with primary (carboxyl) carbon- C^{14} which had a specific activity of 55 mcurie. Its purity was checked by paper chromatography and radioautography. We administered the phenylalanine to the rats subcutaneously and in water solution in doses with activity of 2970 imp/min per 1 g. of body weight (about 40 mcurie/kg or 0.73 mg/kg). We killed the rats by decapitation, 1, 4, 6 and 24 hours after administration of the phenylalanine- C^{14} , and removed the tumors and organs. We took tumors and organs from 3 rats for each test. In order to stop the enzymatic processes we placed the organs at once into a mixture of dry ice and acetone. Then we homogenized pieces of the organs and the tumors and precipitated the proteins with trichloroacetic acid. We washed the precipitates successively with decreasing concentrations of trichloroacetic acid; we removed the lipids with alcohol, and then with a mixture of alcohol and ether, and with ether. After drying the protein we ground it into a fine powder. We measured the radioactivity with an end-type counter of the type MST-17 in a B-1 apparatus. We carried out the determination on 10 mg. samples.

RESULTS

Results obtained are presented in the table and in the graph. As seen in the graph the label is found quite quickly in the proteins of the organs and sarcoma 45 examined by us. The maximum incorporation is reached in the first hours after administration of the phenylalanine- C^{14} . However, on the basis of the intensity of incorporation and the distribution of the label with respect to time, the tumor and the different organs differ from each other.

Phenylalanine is most intensively incorporated into the protein of the kidney. The activity of the proteins of the kidney reach a maximum four hours after administration of the phenylalanine and exceeds the activity of the proteins of the spleen by three times. In the sixth hour, the activity of the proteins of the kidney is somewhat lowered and is then maintained at a steady level during the course of the entire period of the examination (24 hours), exceeding the activity of the protein of all other organs and of the sarcoma 45.

The liver and intestinal wall follow the kidney in the intensity of incorporation. With respect to activity, the proteins of both these organs are one and the same. The highest level of incorporation is reached in the first hour, and is maximum for the wall of the intestine. Some decrease of activity of the proteins of the intestinal wall is noted after four hours; in the course of 6-24 hours, its activity is maintained without change. The activity of the proteins of the liver is somewhat lowered after four hours, is again raised, reaching a maximum after six hours, and then decreases.



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Phenylalanine is less intensely incorporated into the proteins of the spleen, in comparison to the organs cited above. As seen in the graph, the curve of incorporation of the label into the proteins of the spleen also differs a little from the curve of incorporation into the proteins of other organs. The activity increases gradually and is maintained at a specific level from 4 to 24 hours.

Sarcoma 45 approaches the intestine and liver with respect to the intensity of incorporation of phenylalanine into its proteins, but the activity of the tumor does not increase as quickly and is maintained more steadily than the activity of the liver. In another experiment we observed more intensive incorporation of phenylalanine- C^{14} in the course of 6-48 hours, but the label was also retained for a long time at a constant level.

Our results on the distribution of the label after administration in the proteins of the organs agree essentially with the literature reports on the distribution of other aminoacids – for example, glycine [11, 12, 14, 16], tyrosine [15, 18], methionine [1, 6, 8, 10, 13, 17], lysine [19], etc. It appears to be a general rule in the distribution of the marked aminoacids, that in organs such as the kidney, liver, intestinal lining and spleen, an intensive incorporation of the different aminoacids is found. On the other hand, in the muscles, testicles and brain only slight activity is observed. Different tumors have an activity approaching that of the kidney or liver. However, the order of activity in the different organs and tumors is not always maintained, with respect to the various aminoacids.

It was established in our tests that after administration of the phenylalanine- C^{14} the marked isotope is held in the proteins of the tumor for quite a long time, since it was shown that the radioactivity of the proteins of the tumor is decreased more slowly than the proteins of the other organs, for example, the liver or kidney [14, 16].

In agreement with literature reports [1, 5, 8, 13, 15, 16, 17] we did not observe a preferential incorporation of phenylalanine- C^{14} into the proteins of the tumor (sarcoma 45) in comparison with the proteins of the normal organs. However, in the opinion of G. Lepage [14] if the calculation is made, not on the unit of weight of the protein (specific activity), but converted to all of the tumor, then it appears that the activity of the tumor rises to the extent of its growth, whereas the general activity of the other organs decreases.

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No. of test	Time, hrs when exptl. animal was killed after administration of the phenylalanine	Activity of the proteins (in imp/min per 10 mg. dry protein)				
		sarcoma 45 tumor	kidney	liver	intestinal wall	spleen
1	1	65	120	80	98	38
2	4	97	186	58	71	57
3	6	80	128	95	82	59
4	24	78	152	42	88	60

As we showed earlier [7], the different intertwists of the tumor differ significantly one from the other on the basis of the intensity of the incorporation of the marked aminoacids into the proteins. Therefore, with respect to aminoacid metabolism, it appears to us that it is better to compare the organs, not with tumors in general, but only with a specific tumor.

It is interesting that the incorporation of phenylalanine- C^{14} into the proteins of sarcoma 45 and of the organs of rats (in our results) is quite similar in general characteristics to the distribution of labeled sarcosyl (p-di-2-chloro-ethyl) aminophenylalanine- C^{14} in the proteins of these same organs and tumor [3].

SUMMARY

Studies of the incorporation of carbon-labelled phenylalanine- C^{14} into the proteins of sarcoma 45 and various organs of the rat were made. It was shown that the label was taken up rather intensively by the tumor and the organs under study (liver, kidneys, intestinal wall and spleen). In the whole period of study the proteins of the kidney contained the highest amount of radioactivity. The specific activity of the proteins of the tumour (sarcoma 45) was near to that of the liver and intestinal wall. However, the label in the tumour maintained on a relatively constant level without lowering in the period of study (24 hours) whereas the radioactivity in the liver was descending steeply. It was interesting to note that there was some similarity between the incorporation of phenylalanine into the proteins of sarcoma 45 and various organs and the distribution of sarcosyl (p-di-2-chloro-ethyl-amino-DL-phenylalanine) in the proteins of these organs and the tumour.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
