Activities of Cystathionase, Cysteine Sulphinic Acid Decarboxylase and Serine Dehydrase in the Liver of Tumour-Bearing Rats

It can be anticipated that disorders in the metabolism of amino acids, proteins, carbohydrates and nucleic acids occur in the tissues of tumour-bearing animals. Since the general review[1] devoted to the tumour-host relations and, especially, to the declines of catalase in the non-neoplastic tissues of the tumour-bearing rats, noticeable changes in some enzymic activities have been observed in the liver of the host animal: on the one hand, all the activities of the NADPH-linked electrons transport system[2], tryptophane pyrrolase[3,4], serine dehydrase[5,6], fructose 1–6 diphosphate phosphatase[5] were decreased after implantation of tumours; on the other hand, acid phosphatase[7], alkaline phosphatase[8], pyruvate kinase[5,9], hexose kinase[5] and phospho-fructokinase[5] were increased, and phenylalanine hydroxylase was not modified[10].

SUDA et al.[5] concluded that in tumour-bearing animals, the liver function is turning on glycolysis and turning down gluconeogenesis. As we have previously observed[6] that the levels of 3 pyridoxal phosphate-dependent enzymes, namely cystathionase (L-homoserine hydrolyase E.C.4.2.1.15), cysteine sulphinic acid (CSA) decarboxylase (L-cysteine sulphinate carboxylyase E.C.4.1.1.29) and serine dehydrase (L-serine hydrolyase E.C.4.2.1.13) were decreased in the liver of rats bearing Guerin epithelomia or a sarcoma of the stomach[11], we have therefore investigated in more detail the effects of tumour implantation on these enzymes: 2 at least, cystathionase and serine dehydrase, intervene in gluconeogenesis. The present paper deals with the results so far obtained.

The experiments were performed on male Wistar rats, with body weight of approximately 160 g, obtained from the 'Centre de Sélection des animaux de laboratoires du C.N.R.S.' and maintained on a commercial diet (UAR entretien). In the first experiment, a group of rats had fragment of the Guerin epithelomia implanted under the skin in the lumbar region. At regular intervals of time after surgery, normal rats (untreated, used as controls) and tumour-bearing animals were killed and bled. The livers were removed immediately, weighed and placed on ice. They were homogenized in a solution of sucrose $0.25M$ (3 ml for 1 g of liver) and the homogenates were centrifuged at 105,000 g for 45 min in a refrigerated Spinco Model L ultracentrifuge. The supernatants were assayed for CSA decarboxylase, cystathionase and serine dehydrase activities, the estimations being carried out, in the presence of pyridoxal phosphate, by the respective methods already described [12–14]. Table I shows the data of a representative experiment.

The results presented above indicate that the activities of the enzymes in the liver of tumour-bearing rats are considerably lower than those of the control animals, which furthermore remain respectively constant during the duration of the experiment. Moreover the decrease in activity occurs rapidly from 7 days after the implantation of the tumour, the levels of the enzymes are approximately half those of the control animals. It is worthwhile to note that the activity of cystathionase in the tumour-bearing rat appears to have reached a plateau after 7 days and then remains virtually constant, whereas the depressions of cysteine sulphinic decarboxylase and of serine dehydrase increase with the time. While our experiments were in progress a report was published[15] according to which the activity of cysteic acid decarboxylase was decreased in the liver of Guerin epithelomia-bearing rat and completely disappeared about 34 days after the implantation of the tumour. As there is good evidence[16] that, in the liver of the rat, a single protein catalyzes the decarboxylation of cysteine sulphinic acid and the decarboxylation of cysteic acid, this result is in good agreement with our observations.

In a second group of experiments, rats were implanted with the sarcoma of the stomach. It was of interest to

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Table I. Enzyme activities in the liver of Guerin-epithelomia-bearing rats

Treatment and No. of animals	Weight of liver (g) (mean)	Cystathionase (μmol H_2S/g fresh liver/h)		CSA decarboxylase (μl CO_2/g fresh liver/h)		Serine dehydrase (μmol pyruvate/g fresh liver/h)	
C [11]	10.3	33.4 ± 8.9[a]		1892 ± 71		184 ± 28	
Tu (7 days) [3]	7.9	17.6 ± 1.0	−46%	858 ± 12	−55%	50.3 ± 7.7	−73%
Tu (15 days) [3]	11.1	18.9 ± 0.5	−44%	740 ± 53	−61%	28.6 ± 3.8	−85%
Tu (22 days) [3]	11.2	15.4 ± 0.2	−54%	580 ± 115	−69.5%	13 ± 1.3	−93%
Tu (43 days) [2]	13.8	18.0 ± 9.2	−46.5%	211 ± 18	−89%		

C = controls; Tu = tumour-bearing rats implanted from the number of days indicated. Figures in brackets are number of animals. [a] Standard error.

determine if another kind of tumour was also effective as 'modifier' of the enzyme activities. Furthermore this sarcoma, unlike Guerin epithelomia, does not develope metastasis, so that it is possible to suppress the pathological status of the animal by surgical removal of the tumour at a definite time after implantation. In the event of decreased activities of the enzymes in the liver of rats bearing this tumour, such a property affords a means of studying whether these decreases are reversible or not, that is to say whether the levels of the enzymes are or are not more or less restored to a control level following removal of the tumour. As results similar to those reported for the Guerin epithelomia-bearing rats were obtained with animals bearing the sarcoma, the removal of this tumour was carried out in a group of rats implanted from 15 days. The results of a representative experiment in which cystathionase and CSA decarboxylase activities were measured are shown in Table II.

Table II. Enzyme activities in the liver of stomach sarcoma-bearing rats and the liver of rats killed 15 days after removal of the tumour

Treatment and No. of animals	Weight of liver (g) (mean)	Cystathionase (μmol H_2S/g fresh liver/h)		CSA decarboxylase (μl CO_2/g fresh liver/h)	
C [10]	8.9	32 ± 2.5		2080 ± 82	
Tu (15 days) [6]	8.7	15 ± 1.75	-53%	1146 ± 103	-45%
Re (15 days) [8]	9.0	31 ± 1.42	-0%	1679 ± 121	-19%

C = controls; Tu (15 days) = tumour bearing-rats killed 15 days after implantation of the sarcoma; Re = rats which beared the sarcoma for 15 days, then the tumour has been extracted and the animals killed 15 days after removal of the tumour.

From this table, it appears on the one hand that the levels of cystathionase and of CSA decarboxylase are decreased by approximately 50% in the liver of rats bearing the tumour from 15 days. On the other hand, removal of the tumour at that time, when the levels of the enzymes are half the control values, provokes recoveries of the enzymes. Thus, 2 weeks after the removal of the tumour, the activity of cystathionase is completely restored, whereas the activity of CSA decarboxylase, although not fully restored, is however significantly increased.

The conclusions that could be drawn from these observations are the following: the levels of the enzymes are noticeably decreased in the liver of the host animal, and essentially similar results were obtained with both tumours. Moreover, the removal of the tumour, when performed, induces significant recoveries of the enzyme activities. As the measures of activity were always carried out with pyridoxal phosphate added to the incubation mixtures, the decrease of activity probably reflects decrease of the quantity of apoenzymes. At the moment it needs more experimentation definitely to decide whether the decrease of these proteins may be attributed to a decrease of their rate of biosynthesis or to an increase of their rate of degradation[17].

Résumé. Dans le foie de rats porteurs de tumeurs (épithéliome de Guérin et sarcome de l'estomac), les activités de la cystathionase, de la décarboxylase de l'acide cysteine sulfinique et de la sérine déhydrase sont considérablement diminuées: cette diminution est de l'ordre de 50%, 15 jours après implantation de ces tumeurs. Si à ce moment le sarcome (qui ne provoque pas de métastases) est chirurgicalement extirpé, on observe une restauration des activités enzymatiques qui, après 15 jours, est totale pour la cystathionase et très significative pour la décarboxylase.

FERNANDE CHATAGNER, A. HIRSCH, ODILE DURIEU-TRAUTMANN and MARIE-CHRISTINE RAIN[18]

Laboratoire de Chimie Biologique, 96 Boulevard Raspail, Paris 6ème (France) and Institut d'Immuno-Biologie de l'INSERM et de l'Association Claude Bernard, Hopital Broussais, Paris 14ème (France), 21 April 1969.

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[18] Equipe de Recherche C.N.R.S. No. 43.

Cyanocobalamine as a Support of the in vitro Cell Growth-Promoting Activity of Serum Proteins

Whether primary tissue explantation or cell strain maintenance is concerned, most culture media usually contain serum[1] and sometimes proteic[2] or tissular extracts[3]. When proteins are completely absent, mammalian cells do not survive for long, except in a very few cases where cells have been specially adapted[2,4]. In the less well-known field of insect tissue culture, cell growth and cell multiplication are, under these conditions, only exceptionally observed in vitro. Since almost all currently used culture media were based on the physico-chemical analysis of insect hemolymph, as far as the inorganic salt and amino acid composition is concerned, it would be tempting to relate the remaining difficulties to the kind of protein supplementation of the medium. Indeed, only 2 satisfying supplements have been described: in the first case[5], about 4% of heat-treated hemolymph from *Antheraea pernyi* diapausing pupae is added to the culture medium; in the other cases, adopted by almost all