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.

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[17] The technical assistance of Mmes ANNE-MARIE LORINET-CROC, YVELINE VAN HEIJENOORT, YVELINE GICQUEL and Mlle CHRISTIANE PORTEMER is gratefully acknowledged.

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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

workers[6], about 10% of fetal calf serum is used as an additive.

Recently, we succeeded in cultivating an insect cell line in a serum-free medium[7], called S19, already described, which included only certain human serum protein fractions. The separate use of these fractions enabled us to distinguish 2 different but complementary effects[8]: (1) a cell-protecting effect which could be provided by serum antiproteases, e.g. α2-macroglobulin, and (2) a cell-growth promoting activity correlated with human serum fraction V and which we tried to elucidate.

The following experiments have been made with *Periplaneta americana* L fibroblastic cells (EPa strain)[6]. Two cell cultures have been developed separately: cells (E1), arising from the initial EPa strain, regularly maintained in a medium containing fetal calf serum[6], and cells (E2) cultivated for 3 months in the serum-free medium S19[7] to avoid any subsequent interference with the effect of fetal calf serum. About 2×10^6 cells were inoculated in 50 ml tightly closed flasks containing 4 ml of culture medium. The water-jacketed incubator was adjusted to 30 °C.

We first studied the modalities of the cell-growth promoting activity of human serum fraction V. In the same but 'albumin-free' medium S19, the cell cultures changed to a slower growth rate and degenerated a few weeks later. It proved necessary to add 4 g/l of human serum fraction V to obtain the expected growth-promoting effect on these cells. The high amount of the protein supplement needed was not to be understood, as the analysis of the metabolized culture media did not reflect any protein utilization by the cells[7]. Moreover, an equivalent quantity of crystallized (pure) albumin proved to be inefficient. Thus crude fraction V contains an active factor absent from purified albumin. As α1-globulins are the main contaminants of fraction V, the activity might be associated with these proteins[9]. α1-anti-trypsin and α1-acid glycoprotein are the most important proteins of the α1-globulin group, but the cell growth-promoting activity is not associated with the cell-protecting effect provided by antiproteases. A purified sample of α1-acid glycoprotein was found to be devoid of any activity. Thus, the activity seems to be supported by one of the minor components of the α1-globulin group. However, as for mammalian cell lines, we succeeded with our insect cell strain in substituting an enzymatic lactalbumin hydrolysate (5 g/l) for fraction V. This fact allows for the exclusion of a specific action of serum proteins and consequently of specific carrier functions which might be attributed to certain α1-globulins. Therefore, the observed effect of fraction V may eventually be attributed to some low molecular components carried by the protein macromolecules.

The active substance might well be a vitamin, and vitamin B_{12} must be specially considered with respect to tissue culture. Indeed, several investigators have reported experiments with certain cell strains needing vitamin B_{12} for maximal cell growth[2,4]. Vitamin B_{12} is normally bound to serum proteins and has been found in all 6 Cohn plasma fractions, although the highest percentage was associated with fraction V[10]. More recently, non-specific binding of vitamin B_{12} could be distinguished from specific binding and 2 carrier proteins called transcobalamin I and II could be identified[11]. Transcobalamin I is an α1-globulin and has the properties of a seromucoid. On account of the very low serum concentration of these proteins, cyanocobalamine itself must be active in an infinitesimal rate. Consequently, crystallized vitamin B_{12} (Calbiochem) has been added to our culture medium S19, free of serum fraction V, in 3 different concentrations: 10 γ/l, 1 γ/l and 0.1 γ/l. All these media were tested on EPa cells. E1 cells, as well as E2 cells, showed an active growth and multiplication rate. Subsequent subcultures being normally successful, no anomaly was observed for several months. The optimal concentration of vitamin B_{12} in the culture medium seemed to be located between 1 and 0.1 γ/l. This fact is in agreement with the requirements for hydrosoluble vitamins of EPa cells[12]. In very slow-growing cell cultures (E3 cells, deprived both of fetal calf serum for 3 months, and of human fraction V for 1 week) lack of cell multiplication is first observed, rapidly followed by lethal cell damages. However, E3 cells recover their activity in less than 2 weeks after cyanocobalamine supplementing of the same medium. Thus, in the absence of vitamin B_{12}, no cell survival occurs.

The biochemical significance of cyanocobalamine in cell metabolism is still not clearly defined. A competitive action with folic acid is sometimes noticed in nucleic acid and protein synthesis[4,13]. Vitamin B_{12} can also participate in many enzymatic reactions as an electron donor-acceptor[14], like an adenosyl coenzyme[15], especially in the metabolism of methyl groups. The involvement of cyanocobalamine in methionine biosynthesis[16] must be taken into account with respect to the elevated requirements of insect cells for sulphur amino-acids[7].

Résumé. La mise au point d'un milieu synthétique pour cellules d'insectes a permis de caractériser deux actions complémentaires des protéines du sérum qu'il fallait jusqu'alors ajouter au milieu de culture. La première, revient à une protection des cellules par les antiprotéases du sérum, notamment à l'occasion de leur repiquage. L'autre, objet de ce travail, établit le rôle de certaines protéines-transport, et plus particulièrement de celles servant de support à la vitamine B_{12}.

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