Chapter IV

Changes in the amino acid composition of the culture medium during cultivation of normal, neoplastic, newborn, and regenerating liver

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The amino acid requirements of tissue cultures have been studied in a variety of cultures of explants as well as cell lines in synthetic and semi-synthetic media (164,187-192). Such studies have pointed to the distinguishing characteristics of a few cultures, such as the non-requirement of glutamine by chick heart (154), the requirement of serine by rabbit fibroblasts (156), and of asparagine by Walker carcinosarcoma 256 (155).

The presence of similar distinguishing characteristics in amino acid requirement between normal and neoplastic liver cultures was investigated in the present experiments. Comparative data were obtained simultaneously on newborn and regenerating liver. The details of these investigations are given in this chapter.

Experimental

Procedures for the preparation and cultivation of the cultures and details of equipment and chemicals used have been described in Chapter II.

Additional use was made of the following materials for the chromatographic estimation of the amino acids:

acetone, n-butanol, phenol, ethanol, glacial acetic acid, boric acid and ninhydrin.

Estimation of amino-acids in the culture fluid

The large number of samples collected during the experiments necessitated the use of a rapid method that would at the same time be sufficiently quantitative. Previous studies in this laboratory had shown that the circular paper chromatographic methods described by Giri and his associates (193-195) as used by Gothoskar *et al.* (196) can be used with advantage to separate and estimate many of the amino acids in both fresh and used media. This method was therefore followed except that the phenol solvent used for the separation of aspartic acid, glutamic acid, serine, glycine and threonine was replaced by phenol-borate buffer system as described by Wade *et al.* (197) and that chromatographic papers were used without washing.

The amount of amino acid or amino acid mixture present in the aliquot spotted was determined and from this the amount present in 100 ml. of the medium was calculated. The determinations were made after 72 hours of cultivation as this corresponds to the period of maximum metabolic activity.

Results and discussion

The changes in the amino acid content of the medium after a 72 hour period of cultivation are shown in Table 5. Fig. 3 shows the same with regard to arginine, alanine, glutamine, glutamic acid and threonine. It can be seen from the table that the pattern of changes shows some common as well as distinguishing features between the different cultures. All the cultures are found to release into the medium glutamic acid, serine, lysine + histidine, and leucine + isoleucine. To this list must perhaps be added glycine and alanine if not for the fact that the change in the case of neoplastic cultures is almost negligible although it is still in the same direction. Similarly, all the cultures are found to utilize arginine, glutamine and aspartic acid, the last mentioned only to a small extent by the hepatoma cultures.

Hepatoma cultures seem to differ from the others in that they utilize threonine, and tyrosine + tryptophane in contrast to other fast-growing tissues as well as normal liver. The removal of threonine from the medium by the hepatoma cultures in contrast to the opposite phenomenon exhibited by the other cultures is suggestive of underlying differences in the metabolism of threonine and/or tissue levels of this amino acid. A similar suggestion also arises in regard to alanine, tyrosine + tryptophane and aspartic acid.

Table 5

The amino acid composition of the medium during cultivation of normal, neoplastic, newborn and regenerating liver

Amino acid	Amino acid content of the medium (mg./100 ml.)				
	Initial	After cultivation for 72 hours			
		Liver tissues cultivated			
		Normal	Neoplastic	Newborn	Regenerating
Aspartic acid	12.0	8.1	11.0	7.5	8.3
Glutamic acid	15.0	20.4	18.7	27.3	20.0
Serine	5.0	7.5	8.0	5.5	6.5
Glycine	5.0	8.1	5.5	6.1	7.6
Threonine	15.0	19.0	9.1	16.2	14.8
Lysine + Histidine	39.0	47.0	52.0	50.0	49.0
Arginine	30.0	0.6	20.4	0.2	0.3
Glutamine	35.0	28.9	23.0	17.5	14.0
Alanine	5.0	11.0	6.0	11.6	10.0
Tyrosine + Tryptophane	12.0	11.2	8.7	13.0	12.5
Valine + Methionine	23.0	22.0	22.0	18.6	22.0
Phenylalanine	10.0	11.6	10.0	8.5	11.0
Leucine + Isoleucine	10.0	15.0	13.0	12.5	12.0

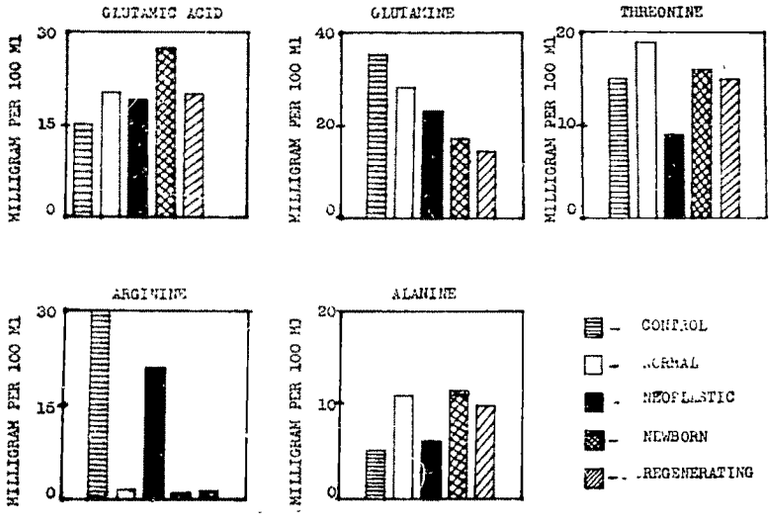

Fig. 3. Changes in the concentration of certain amino acids of the medium during cultivation of different liver tissues.

The difference in the utilization of arginine is interesting in the light of the suggestion that the capacity to catabolize arginine is diminished in the liver of rats fed with azo-dye as judged from the observation that urea formation from arginine by these liver tissues is markedly reduced (141c). That this may be due to a low level of arginase in this tissue is suggested by the observation of lowered levels of this enzyme in Novikoff hepatoma (198).

Similarly, the cultures of newborn liver appear to differ from other tissues in regard to the rate of utilization of phenylalanine, and valine+methionine and that of release into the medium of glutamic acid.

Inspite of the qualitative similarity of the cultures in regard to the utilization of arginine, glutamine and alanine, some differences emerge on closer examination of the data. To elaborate, the cultures of normal, regenerating and newborn liver tissue are seen to use up almost all the arginine present in the medium whereas barely a third of the amount is utilized by the neoplastic cultures. On the other hand, the rate of utilization of glutamine by these cultures would appear to be higher than that in the normal cultures. Similarly, the release of alanine into the medium by the hepatoma cultures is negligible as compared to that by the other cultures.

With regard to the increased rate of glutamine utilization by hepatoma as compared to normal liver, it must be pointed out that a similar observation has been made in the case of other varieties of malignant cells cultivated *in vitro* (188,191,192). This would also appear to be consistent with the general finding that plasma, liver, and muscle levels of this amide are found to be lowered in tumor-bearing rats. This would be suggestive of underlying differences in glutamine metabolism.

Thus the pattern of changes in the amino acid content of the medium with the cultivation of these tissues is such as to suggest underlying differences between hepatoma and normal liver with regard to the metabolism of a number of amino acids. Such a suggestion is reinforced in the case of arginine and glutamine by other related findings.