Amino Acids in Epidermal Carcinogenesis in Mice*†

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The present investigation is one of a series dealing with the nitrogen metabolism of mouse epidermis in various phases of growth and in carcinogenesis (1 to 4). The content of 12 amino acids was determined in dried ground samples of normal epidermis and epidermis of mice receiving varying numbers of standardized applications of methylcholanthrene (MC) in benzene (0.6 gm. in 88 gm. of benzene) or of benzene alone. A transplantable squamous cell carcinoma (Tumor I), originally derived from a carcinoma produced on the back of a mouse by the application of MC (5), was also studied.

MATERIALS AND METHODS

The methods employed for the treatment of the animals and for the preparation of the tissues have been described previously (6). The pooled tissues were dried rapidly to constant weight *in vacuo* over P_2O_5 prior to hydrolysis and the water content calculated. Five individual pooled samples of normal epidermis were analyzed, four tumor samples, two samples each of tissues receiving 1, 6, 12, and 24 paintings of the carcinogen, and one sample of all of the other tissues reported. Material obtained from approximately 1500 mice was used in these studies. Nitrogen determinations were performed in duplicate or triplicate by a micro-Kjeldahl procedure.

The animo acids were determined in hydrolysates of the whole tissue. Acid hydrolysis was employed for all of the amino acids except tryptophan, for the determinations of which an enzymatic digest was made with pancreatin. Leucine, valine, isoleucine, cystine, methionine, lysine, phenylalanine, histidine, arginine, and threonine were determined by adaptations of the techniques of Barton-Wright (7). Glutamic acid was assayed according to the method of Hac, *et al.* (8), but the samples were prepared for analysis as outlined by Barton-Wright (7). The tryptophan content was measured according to Greene and Black (9), but the basal medium was modified to conform in part to that of the U.S.P. XIII nicotinic acid test.

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The results are expressed in mg. of amino acid per 100 mg. of dry weight of tissue or mg. of amino acid nitrogen per 100 mg. of tissue nitrogen. The latter method is preferable since variations in non-nitrogenous constituents such as fat or glycogen can affect the former calculation. In general, the results showed the same trends by either method of calculation. Comparisons of the values for the various amino acids expressed on the total nitrogen basis among the different groups of animals were made by the use of analysis of variance (10). There were no statistically significant correlations of the number of treatments with the degree of change in the constituents studied. It was, therefore, possible to combine the values for the tissues receiving different numbers of paintings with pure benzene or with the carcinogen in single means. The values for the epidermis of mice receiving 3 paintings with MC are also listed separately because the changes after this treatment seemed more marked than in the other carcinogen-treated groups.

DISCUSSION OF RESULTS

Water, nitrogen, and total amino acid contents.— The water content of the epidermis increased on treatment with the carcinogen and showed a further increase in the carcinoma, in confirmation of previous work (11) (Table 1). On the other hand, benzene alone produced definite decreases after 12 and 24 paintings.

There was an increase in the nitrogen content of the tissues after treatment with MC when the results were expressed in percentage of dry weight, and a return to normal in the tumors. There was a slight decrease in the benzene-treated tissues. These changes in the N:weight ratio are for the most part accounted for by altered lipid contents.[1]

The sums of the 12 amino acids showed highly significant increases as a result of the treatment with MC and in the tumors by either method of calculation. If it can be assumed that the undetermined amino acids showed a trend similar to the 12 that were determined, the data can be taken to indicate a decrease in non-amino acid nitrogen relative to amino acid nitrogen in the MC-treated epi-

[1] Unpublished findings.

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dermis and in the tumors. This is supported by the decreases in urea and ammonia which were found previously (4). Benzene treatment produced a smaller relative increase in amino acid nitrogen.

Changes in amino acids produced by the application of benzene.—The levels of lysine, isoleucine, leucine, phenylalanine, threonine, histidine, and glutamic acid were not significantly altered by painting with pure benzene ($P > 0.05$) (Table 2). The arginine, tryptophan, and valine contents were elevated ($0.01 < P < 0.05$). Cystine was higher in the epidermis of the treated animals than in the normal controls ($P < 0.01$), and the methionine was significantly lower ($P < 0.01$). The influence of benzene on the sulfur amino acids is of special interest. Two of the ways in which benzene is known to be detoxified by some species are as the *l*-phenylmercapturic acid, and following oxidation to phenolic derivatives, as phenylsulfuric acids (12). The sulfur-containing residues of these derivatives must come from cystine or methionine or both. The disturbance in the methionine and cystine levels of the epidermis after application of benzene suggests that the epidermis might perform detoxications similar to those ascribed to the liver. In this connection it is also interesting that the taurine level of epidermis is decreased by the application of benzene (3).

Changes in amino acids produced by the application of MC in benzene.—The contents of arginine ($P < 0.01$), valine ($0.01 < P < 0.05$), and cystine ($P < 0.01$) were higher in the epidermis of this group of animals than in the untreated controls, but the values were not significantly different from those of the benzene-painted mice. On the other hand, the methionine content increased over the normal value ($0.01 < P < 0.05$) after treatment with the carcinogen, as contrasted with the decrease in this amino acid produced by benzene alone, the difference between the means of the solvent-treated and MC-treated groups being highly significant ($P < 0.01$). The tryptophan content was higher than in the normal animals and lower than in those treated with benzene, but the differences were not within the 5 per cent range of significance. The values for histidine, isoleucine, phenylalanine, leucine, and lysine were higher after MC-application than either in the normal or in the benzene-painted mice, all the P-values being below 0.05. It is evident from the above discussion that the changes in the amino acid content of epidermis produced by the application of a benzene solution of methylcholanthrene are different from those produced by the solvent alone.

Amino acid pattern in the transplantable squamous cell carcinoma.—The levels for the leucine, isoleucine, lysine, methionine, valine, phenylalanine, and threonine in the tumors were significantly higher than in the epidermis of the untreated or benzene-painted mice, the P-values being less than 0.01 for all comparisons except for the threonine content in the solvent-treated mice, in which case the P-value was between 0.01 and 0.05. However, when the mean values for the above 7 amino acids in the carcinomas were compared with the means in the epidermis from MC-painted mice, only the lysine, leucine, and valine contents were found to be higher in the tumors ($0.01 < P < 0.05$). The levels of all seven of these amino acids in the epidermis of mice painted thrice with the carcinogen were very similar to those for the carcinomata. The tumors showed an elevation of tryptophan over normal epidermis ($0.01 < P < 0.05$), but similar or greater increases were found in epidermis receiving 6, 12, and 24 applications of benzene and 1 and 18 treatments with MC. The cystine and arginine contents of the carcinomata were lower than in the benzene- or carcinogen-treated series ($0.01 < P < 0.05$), but were virtually identical with the values found in normal epidermis. The glutamic acid and histidine contents of the tumors were not significantly different from those of the other groups.

GENERAL COMMENT

The chief purpose of the present investigation was to determine whether a change occurs in the over-all amino acid distribution when normal epidermis is transformed to a malignant tumor. The comparison in answering this question must be made not only between the normal epidermis and the tumor but also between the tumor and the precancerous hyperplastic tissue. Although the latter tissue, from foci in which tumors eventually arise, frequently differs markedly from normal epidermis with respect to the variables studied (13), it possesses none of the characteristics of malignancy. In previously reported work on nitrogen metabolism in epidermal carcinogenesis (4) it was found that of a series of tissues painted different numbers of times with MC, the epidermis receiving 3 applications was more like the tumors with respect to arginase activity and the percentage of total nitrogen found in the trichloroacetic acid-soluble fraction than samples of epidermis receiving greater numbers of paintings of the carcinogen. The latter findings, together with an examination of the weight changes of the tissues (4), suggested that the rate of protein synthesis might be greater in epidermis as a whole after 3 paintings than after subsequent applications of the MC. It is interesting that in the present study the thrice-painted

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TABLE 1

WATER, NITROGEN, AND TOTAL AMINO ACID CONTENTS

TISSUE	WATER CONTENT Per cent of fresh weight		NITROGEN CONTENT Per cent of dry weight		AMINO ACIDS DETERMINED Per cent of dry weight		N as per cent of total N	
Normal*	59.1 (58.1–60.0)		11.62 (11.27–12.08)		35.58 (35.27–36.27)		44.83 (44.44–45.35)	
	MC§	Benzene	MC	Benzene	MC	Benzene	MC	Benzene
1 painting‡	60.3	57.3	12.26	11.27	40.78	37.42	49.34	49.91
3 paintings	62.5	60.3	12.43	11.07	49.00	38.34	55.50	50.83
6 paintings‡	63.4	59.7	13.27	11.27	46.83	36.71	50.92	46.73
12 paintings‡	63.4	54.0	12.97	10.62	43.79	34.49	49.24	46.64
18 paintings	64.0		13.33		48.35		52.50	
24 paintings‡	62.2	52.5	12.78	10.76	48.46	34.13	54.76	47.23
		81.6		11.70		43.75		52.89
Tumor†		(81.2–82.0)		(11.00–12.53)		(39.37–44.55)		(47.50–53.95)

* Five determinations.
† Four determinations.
‡ Two determinations.
§ Methylcholanthrene.

TABLE 2

CONTENT OF INDIVIDUAL AMINO ACIDS

AMINO ACID	NORMAL*		BENZENE†		BENZENE+MC‡ (ALL SAMPLES)		BENZENE+MC§ (3 PAINTINGS)		CARCINOMAS‖	
	Mg. per 100 mg. dry wt.	Mg. N per 100 mg. total N	Mg. per 100 mg. dry wt.	Mg. N per 100 mg. total N	Mg. per 100 mg. dry wt.	Mg. N per 100 mg. total N	Mg. per 100 mg. dry wt.	Mg. N per 100 mg. total N	Mg. per 100 mg. dry wt.	Mg. N per 100 mg. total N
Lysine	3.05 (2.68–3.47)	5.15 (4.25– 5.85)	2.91 (2.67–3.22)	5.08 (4.81– 5.53)	4.14 (3.25– 5.32)	6.29 (5.69– 8.21)	5.32	8.21	4.97 (4.39–5.76)	8.18 (7.10– 8.81)
Isoleucine	2.82 (2.56–2.93)	2.65 (2.44– 2.88)	2.65 (2.43– 2.96)	2.58 (2.31– 2.86)	3.91 (3.31– 4.48)	3.26 (2.98– 3.84)	4.48	3.84	4.19 (4.09–4.32)	3.83 (3.48– 4.06)
Leucine	4.44 (4.17–4.87)	4.17 (3.79– 4.58)	4.30 (3.91– 4.84)	4.19 (3.76– 4.68)	5.64 (5.38– 6.13)	4.69 (4.41– 5.26)	6.13	5.26	5.78 (5.34–6.09)	5.28 (5.19– 5.45)
Methionine	1.09 (1.04–1.13)	0.91 (0.87– 0.95)	0.86 (0.74– 1.03)	0.74 (0.65– 0.88)	1.47 (1.19– 1.78)	1.09 (0.93– 1.36)	1.78	1.36	1.48 (1.44–1.52)	1.20 (1.16– 1.24)
Valine	2.71 (2.26–2.89)	2.84 (2.56– 3.04)	2.80 (2.72– 3.00)	3.05 (2.90– 3.18)	3.48 (3.04– 3.76)	3.23 (2.92– 3.52)	3.66	3.51	3.77 (3.56–3.92)	3.86 (3.40– 4.25)
Phenylalanine	1.96 (1.92–2.03)	1.46 (1.38– 1.57)	1.80 (1.69– 1.92)	1.39 (1.29– 1.53)	2.70 (2.20– 2.94)	1.95 (1.53– 2.86)	2.94	2.00	2.60 (2.56–2.63)	1.89 (1.78– 1.98)
Threonine	2.37 (2.21–2.53)	2.43 (2.35– 2.56)	2.11 (1.90– 2.82)	2.26 (2.02– 2.95)	2.87 (2.34– 3.33)	2.62 (2.24– 3.15)	3.33	3.15	3.15 (2.95–3.86)	3.17 (2.76– 3.83)
Histidine	1.38 (1.31–1.45)	3.33 (3.20– 3.45)	1.32 (1.27– 1.48)	3.24 (3.09– 3.49)	1.70 (1.55– 1.86)	3.57 (3.43– 3.78)	1.62	3.52	1.47 (1.44–1.48)	3.40 (3.10– 3.63)
Glutamic acid	9.43 (9.02–9.72)	7.90 (7.13– 8.64)	9.12 (7.70–10.03)	7.86 (6.80– 8.46)	11.65 (9.64–12.48)	8.65 (7.49– 9.53)	12.42	9.53	9.47 (8.81–9.82)	7.72 (7.20– 8.20)
Cystine	1.47 (1.22–1.73)	1.51 (1.22– 1.78)	2.24 (1.85– 2.72)	2.38 (1.92– 2.86)	2.27 (1.94– 2.91)	2.06 (1.80– 2.64)	2.12	1.98	1.42 (1.34–1.48)	1.42 (1.25– 1.56)
Arginine	4.06 (3.43–4.32)	11.50 (9.70–12.70)	4.72 (4.06– 5.66)	13.79 (11.85–16.15)	5.35 (4.48– 5.91)	13.53 (12.34–14.29)	4.48	12.34	4.10 (3.33–4.63)	11.34 (8.55–13.05)
Tryptophan	0.82 (0.62–1.16)	0.98 (0.75– 1.50)	1.36 (0.82– 1.86)	1.71 (1.05– 2.36)	1.02 (0.72– 1.54)	1.10 (0.78– 1.73)	0.72	0.80	1.35 (1.17–1.73)	1.60 (1.31– 2.16)

* Five samples.
† Five samples.
‡ Ten samples.
§ One sample.
‖ Four samples.

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epidermis showed an excellent agreement with the carcinoma in the content of lysine, isoleucine, leucine, methionine, valine, phenylalanine, threonine, and histidine. The increases in cystine and arginine found in the carcinogen-treated epidermis may have been a result of the effect of the solvent alone, since the benzene-treated tissues showed similar increases. This effect did not carry over to the tumors, which had values close to those found in normal epidermis for these amino acids.

It appears clear from the results that the changes in the distribution of amino acids produced by benzene alone are different from those taking place in carcinogenesis. However, it cannot be said that the tumor tissue as a whole shows a distinctive pattern of amino acids which would set it completely apart from all of the non-malignant hyperplastic epidermal samples.

Changes which take place in the total amino acid content of tissues, such as those reported herein, may result from alterations in the ratios of cell types and quantitative and qualitative changes in intracellular constituents. The characterization of various cell fractions with respect to amino acid content is now under way in this laboratory in an effort to determine the physiological significance of the changes reported in this paper. It is already evident that the changes found in the total amino acids cannot be directly correlated with the changes in free amino acids, since it has been previously shown (3) that there is a general increase in the individual free amino acids over the normal in the hyperplastic epidermis and a marked decrease in the carcinomata.

SUMMARY

1. The contents of lysine, isoleucine, leucine, methionine, valine, phenylalanine, threonine, histidine, glutamic acid, cystine, arginine, and tryptophan were determined by microbiological methods in hydrolysates of dried ground samples of whole normal mouse epidermis, of epidermis of mice receiving varying numbers of standardized applications of methylcholanthrene (MC) in benzene or of benzene alone, and of transplantable squamous cell carcinomata originally derived from a carcinoma produced on the back of a mouse by the application of the carcinogen.

2. The sum of the 12 amino acids showed highly significant increases over normal as a result of the treatment with MC and in the tumors when the results were expressed either in terms of mg. of amino acids per 100 mg. of dry weight of tissue or mg. of amino acid nitrogen per 100 mg. of tissue nitrogen. Benzene treatment produced a smaller relative increase in amino acid nitrogen and no significant change on the dry weight basis.

3. From a consideration of the individual values for the amino acids in the tissues studied it was concluded that benzene alone produced significant changes in amino acid distribution in the epidermis and that more extensive and different alterations were produced when MC in benzene was applied. The distribution of amino acids in the carcinomata was similar in most respects to that found in the precancerous hyperplastic epidermis.

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