The molecular ions of the C_9 and C_{13} esters are of very low relative abundance; the C_{19} and C_{24} are entirely absent. The major peaks in the gas-liquid chromatograms of the esters ranged from C_{15} to C_{23} with a maximum also at C_{17}. There is a large peak due to the C_{16} acid for both the high-resolution mass spectrum and the gas-liquid chromatogram of the branched esters. The major peaks were also collected, and their low-resolution mass spectra were determined. The following acids were identified from the mass spectra of their methyl esters (Figs. 2 and 3): 3,7,11-trimethyldodecanoic acid; 4,8,12-trimethyltridecanoic acid; 5,9,13-trimethyltetradecanoic acid; 2,6,-10,14-tetramethylpentadecanoic acid (norphytanic acid); 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid); and 4,8,12,16-tetramethylheptadecanoic acid. Coinjection of methyl phytanate enhanced its respective peak in the analytical gas-liquid chromatograms.

The aliphatic isoprenoid carbon skeleton has been isolated from carbonaceous sediments of various geologic formations ranging from the early Precambrian to the most recent (*11*), and has been taken as presumptive evidence for the occurrence of the photosynthetic process as far back in terrestrial chronology as 3.2 billion years. The possibility of migration of organic molecules after sediment deposition and compaction has brought up in previous discussions the question whether these molecules are indeed indigenous. McCarthy and Calvin (*12*) have discussed the significance of the molecular structures of hydrocarbons with respect to their possible diagenetic transformations and biological or nonbiological origin. The isolation and identification of any particular molecular species is of debatable significance without the assurance that the species was laid down at the time of deposition and compaction of the sediment.

The occurrence of isoprenoid acids in the extract from the chromic acid oxidation of kerogen concentrate from the Green River Formation is evidence that these acids are bound to the kerogen matrix. This means that they are indigenous and were laid down at the time of sedimentation and thus incorporated into polymeric organic structures. The nature of their linkage to the matrix is still under investigation at this laboratory. It is therefore of fundamental significance to corroborate earlier findings with this evidence. In addition, a knowledge of a particular molecule's structure and abundance, relative to the large number of organic components present in any carbonaceous rock, must be ascertained before formulating hypotheses regarding that particular molecule's diagenesis and significance as a "biological marker." For such reasons, detailed characterization of the acidic components of the solvent extracts of the total organic matter in the Green River Formation has been carried out (*3, 4, 5, 6, 13*). Such findings should catalyze similar studies of carbonaceous sediments as a function of chronologic age.

A. L. BURLINGAME
B. R. SIMONEIT
Department of Chemistry and
Space Sciences Laboratory,
University of California, Berkeley 94720

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Asparagine Synthetase Activity of Mouse Leukemias

Abstract. *Various transplanted leukemias and normal tissues of the mouse were tested for asparagine synthetase activity. Leukemias susceptible to suppression by asparaginase have little or no synthetase activity. In contrast, leukemias insensitive to asparaginase exhibit substantial and often very high asparagine synthetase activity. Asparaginase-resistant variants of sensitive leukemias also have considerable synthetase activity. Thus the requirement by certain malignant cells of exogenous asparagine, which entails sensitivity to asparaginase, may be ascribed to lack of asparagine synthetase. Development of asparaginase-resistant variants from asparaginase-sensitive lines is consistently associated with acquisition of asparagine synthetase activity.*

Kidd's observation (*1*) that administration of guinea pig serum causes regression of certain mouse and rat lymphomas has been confirmed (*2*, *3*). Broome produced evidence that the antitumor activity of guinea pig serum is due to its content of L-asparaginase (*4*); this also has been extensively confirmed (*5*). Thus asparaginase of *Escherichia coli* was shown (*6*) to have an antitumor effect similar to that of asparaginase of guinea pig serum. Lymphoma in the dog (*7*, *8*) and lymphoblastic leukemias in man (*8*, *9*) may respond to therapy with asparaginase. The evidence points to the conclusion that tumors and leukemias that respond to asparaginase depend on an external supply of asparagine, and that when this is hydrolyzed by asparaginase in vitro or in vivo the cells die.

A plausible explanation of the requirement for asparagine by certain malignant cells is that they have lost the capacity to synthesize it. Several pathways of biosynthesis of asparagine have been proposed (*10*). Conversion of aspartate to asparagine in certain microorganisms has been shown to require ammonia and adenosine 5′-triphosphate (ATP) and to be associated with cleavage of ATP to inorganic pyrophosphate and adenosine 5′-monophosphate (*11*). The amide nitrogen atom of glutamine rather than the nitrogen of ammonia is utilized for asparagine synthesis in HeLa cells (*12*), and subsequent work (*13*, *14*) has confirmed the participation of glutamine in the synthesis in vitro of asparagine in mammalian systems. Synthesis of asparagine by preparations of Jensen sarcoma and from nutritional

variants of this tumor in the presence of ATP, glutamine (or ammonia), and aspartate has been described (*13*). The asparagine-requiring parent line synthesized less asparagine than did variant lines that had lost their asparagine requirement apparently as a consequence of culture in an asparagine-free medium.

Independent studies (*14*) of an asparagine synthetase preparation purified from Novikoff hepatoma have shown that synthesis of asparagine is associated with cleavage of ATP; although both glutamine and ammonia (at a much higher concentration) are active as nitrogen donors, there is some variability in their relative effectiveness, depending apparently on the stage of enzyme purification. Tumor asparagine synthetase thus resembles in certain properties several other enzymes that can utilize either glutamine or ammonia as the nitrogen donor (*15*). Asparaginase-resistant and asparaginase-sensitive 6C3HED lymphoma cells utilize about the same amounts of exogenous asparagine for protein synthesis, and asparagine is not converted to aspartate to an appreciable extent in either type of cell; the asparaginase-resistant line synthesizes asparagine at a greater rate than do the asparaginase-sensitive cells (*16*).

We now report experiments on the asparagine synthetase activity of several transplanted mouse leukemias. Normal rat tissues had been found to exhibit extremely low synthetase activity, whereas preparations of Novikoff hepatoma were much more active (*14*). Asparagine synthetase activity is low in normal mouse spleen, lymph node, thymus, liver, and kidney, while activity is greater in brain and testes (Table 1). Seven different asparaginase-sensitive mouse leukemias proved to have no or only slight asparagine synthetase activity (Table 1). It is possible that the low level of synthetase activity found in several leukemias arises from inclusion of normal host cells in the preparation. In contrast, asparaginase-resistant lines derived from these asparaginase-sensitive leukemias exhibited substantial asparagine synthetase activity (Table 1); indeed two of them showed much higher activity than did any of the normal mouse tissues examined. The asparaginase-resistant tumors (Table 1) showed appreciable asparagine synthetase activity, all but three exhibiting levels of synthetase activity considerably higher than did any of the normal tissues studied.

The striking evidence (Table 1) that sensitivity of tumors to asparaginase can be ascribed to lack of the enzyme asparagine synthetase suggests a defined genetic defect, the sensitive leukemia cell being viewed as an auxotrophic mutant. Evolution of resistance to asparaginase by sensitive tumors evidently entails acquisition of the synthetase activity characteristic of normal tissues and of asparagine-independent tumors.

Considering the very high levels of synthetase activity in many resistant tumors, one may ask whether asparagine is performing a special metabolic function in tumors. The only metabolic pathways of asparagine yet known are its utilization for protein synthesis and its conversion to aspartate by hydrolysis or to α-ketosuccinamic acid by transamination (*17*). The possibility that there is a qualitative biochemical difference between tumor and normal tissues in relation to asparagine metabolism merits investigation. It is possible, for example, that in tumor cells asparagine functions in amide nitrogen transfer reactions of the type observed with glutamine (*15*). These considerations suggest therapeutic

Table 1. Asparagine synthetase activity of transplanted mouse leukemias and of normal mouse tissues. Most of these leukemias were induced and carried in mice of our colonies (*18*). For determination of activity, suspensions of viable single cells were prepared from thymus, lymph node, spleen (normal or leukemic), and peritoneal fluid (ascites tumors or leukemias). Cells were washed three times in Earle's balanced salt solution at 4°C and resuspended in three to four times the volume of chilled buffer [0.1*M* tris-HCl (*p*H 7.6), 1m*M* dithiothreitol, 0.5m*M* EDTA]. These cell suspensions or the tissues were homogenized in a Potter-Elvehjem homogenizer with the same buffer. The 105,000*g* supernatants were tested for activity at 37°C in a reaction mixture consisting of 100 μmole of tris-HCl, 5 μmole of Na–ATP, 5 μmole of $MgCl_2$, 20 μmole of L-glutamine, and 0.5 μmole of L-aspartic acid-4-^{14}C (specific activity, 1.67 c/mole) in a final volume of 0.7 ml (*p*H 7.6). The reaction was stopped after 30 minutes by addition of 0.1 ml of 15-percent trichloroacetic acid. After centrifugation, a portion (0.05 ml) was streaked on a Whatman 3-MM paper strip and electrophoresis was carried out at 50 volt/cm for 45 minutes in 0.04*M* sodium acetate buffer (*p*H 5.5). The radioactivity in the asparagine area was determined with a scintillation counter. In all instances it was established by experiments with purified asparaginase (guinea pig) that the radioactivity was due to asparagine. Synthetase activity is given as nanomoles of L-asparagine formed per miligram of protein per hour.

Item	Strain of origin	Mode of induction	Suppression by asparaginase in vivo (*3*)	Asparagine synthetase activity
		Asparaginase-sensitive leukemias		
ERLD	C57BL/6	X-radiation	+	0.4
EARAD1	(C57BL×A)F_1	X-radiation	+	.9
6C3HED*	C3H	Estrogen	+	0
P1798*	BALB/c	Diethylstilbesterol	+	0
ASL1	A	Spontaneous	+	0.7
E♀RL66-7	C57BL/6	X-radiation	+	.3
L5178Y*	DBA/2	Methylcholanthrene	+	0
		Asparaginase-resistant variant lines of asparaginase-sensitive leukemias†		
ERLD-Res-1			—	10
ERLD-Res-2			—	66
EARAD1-Res			—	6.4
6C3HED-Res*			—	34
P1798-Res*			—	5.8
		Asparaginase-resistant leukemias		
RADA1*	A	X-radiation	—	156
BALB♂RL2*	BALB/c	X-radiation	—	39
K36*	AKR	Spontaneous	—	23
ESL1	C57BL/6	Spontaneous	—	2.3
ESL4	C57BL/6	Spontaneous	—	6.0
SJL G1	SJL	Passage A Gross virus	—	13
E♂G2	C57BL/6	Passage A Gross virus	—	27
EL4*	C57BL	Dibenzanthracene	—	55
Meth A (sarcoma)*	BALB/c	Methylcholanthrene	—	65
		Normal tissues‡		
Spleen				2.3
Lymph node + thymus				1.6
Brain				5.2
Testes				13.6
Liver				1.1
Kidney				0.3

* Ascites form; in all other instances cell suspensions were prepared from spleens and lymph nodes of passage mice. † Produced by repeated passage in hosts treated with suboptimal doses of asparaginase, and subsequently carried in untreated hosts. ‡ Average values obtained with tissues from three strains of mice (C57BL×A)F_1♂, C57BL/6♀, BALB/c♂); individual values did not differ substantially from means.

approaches based on exploitation of such a biochemical difference—for example, by selective inhibition of asparagine utilization and biosynthesis in tumors.

BERNARD HOROWITZ
BERTHA K. MADRAS, ALTON MEISTER
Department of Biochemistry, Cornell University Medical College, New York
LLOYD J. OLD, EDWARD A. BOYSE
ELISABETH STOCKERT
Sloan-Kettering Institute for Cancer Research and *Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College*

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Virus-Induced Hemolysis in Erythrocytes Deficient in Glucose-6-phosphate Dehydrogenase

Abstract. *Red cells from individuals deficient in glucose-6-phosphate dehydrogenase undergo increased autohemolysis when incubated in the presence of influenza-A virus. Normal red cells, but not those from individuals deficient in glucose-6-phosphate dehydrogenase, show increased activity of the hexose monophosphate shunt in the presence of the virus. This increase in shunt activity appears to be related to oxidation of cellular sulfhydryl groups.*

Increased hemolysis, as evidenced by jaundice and significant anemia, has been observed in individuals deficient in glucose-6-phosphate dehydrogenase (G-6-PD) and showing clinical signs of viremia and certain bacterial infections in the absence of any possibly offending drug (*1*). The observation that a patient severely deficient in G-6-PD had a recurrent fall in hemoglobin level of at least 2 g during upper respiratory infections prompted this investigation.

The initial experiments were designed to determine whether the hemolytic process associated with viremia could be reproduced in vitro. Red cells from three patients with severe G-6-PD deficiency (less than 10 international units per gram of hemoglobin) were washed three times in isotonic saline and then incubated at 37°C for 48 hours in the presence of serial titers of an influenza virus (*2*). Hemoglobin was measured in the supernatant by a benzidine method (*3*). The values obtained were calculated as percentage hemolysis, as in the autohemolysis test (*4*). In the absence of virus, normal red cells undergo less than 2 percent autohemolysis in 48 hours.

From the results of a typical experiment (Fig. 1) it is evident that concentrations of virus higher than 10^3 particles per cubic millimeter are associated with increased autohemolysis in cells deficient in G-6-PD. The addition of glucose had but little effect on the hemolytic action of the virus. No complement was present, and viral-induced agglutination of the red cells was not observed. Increased numbers of Heinz bodies, however, were observed in cells deficient in G-6-PD incubated with virus. Prior inactivation of the virus by either heat or specific antibody abolished completely the hemolytic activity.

Because this clinical syndrome of hemolysis was associated with a biochemical defect in the hexose monophosphate shunt, the next experiment involved an investigation of this pathway. A relatively simple technique (*5*), for evaluation of this pathway in the intact red cell, uses as an index of hexose monophosphate shunt metabolism the production of $C^{14}O_2$ from glucose labeled in the 1-carbon position. Since in the mature erythrocyte the shunt represents the only known locus

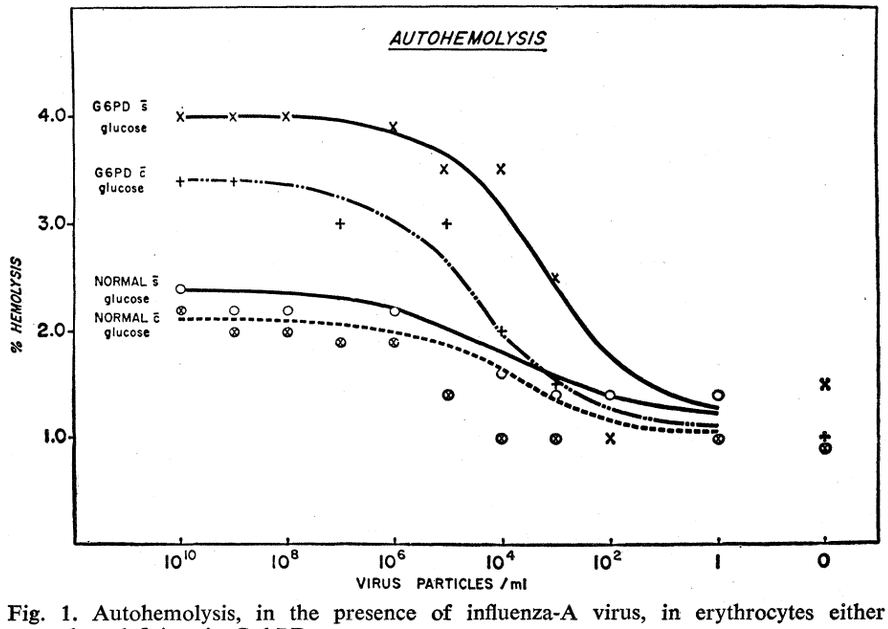

Fig. 1. Autohemolysis, in the presence of influenza-A virus, in erythrocytes either normal or deficient in G-6-PD.