TABLE 1

THE EXCRETION OF LABELED CREATININE AND ALLANTOIN BY CONTROL AND LEUKEMIC MICE IN A 24-HOUR PERIOD AFTER INJECTION OF C^{14}-LABELED SODIUM FORMATE AND C^{14} METHYL-LABELED BETAINE

Substance injected	Total counts injected	Animals	Total creatinine		Allantoin	
			Counts/ min/mouse	Counts/ min/μM	Counts/ min/mouse	Counts/ min/μM
Sodium formate	1×10^6	Control	317	54	1190	80
" "	1×10^6	Leukemic	796	115	2900	142
Betaine	4.7×10^5	Control	656	94	416	20
"	4.7×10^5	Leukemic	913	92	706	21

olism cages. Pooled 24-hr urine samples were collected from each group. For the betaine experiments 10 control and 10 leukemic mice were each injected with 1 mg of betaine hydrobromide (0.53 μc/μM), and urine collections made as in the formate experiments. The urine was autoclaved with acid to convert creatine to creatinine. The creatinine was isolated by carrier isolation and purified as the zinc chloride derivative. Allantoin was isolated by carrier isolation. The total creatinine and allantoin content of the urine before carrier addition was determined in order that specific activity could be determined. The samples were placed on aluminum plates and counted with an end window tube with a window thickness of 2 mg/cm².

In addition to the carrier isolation, the urine samples were subjected to paper chromatography in a phenol water system. The specific activities of creatinine and allantoin as determined by counting the paper strips agreed quite well with the results obtained by carrier isolation procedures.

The results presented in Table 1 show that the leukemic mice excreted more labeled creatinine and allantoin after injection of labeled sodium formate or betaine than did the controls. There is adequate evidence that animals can synthesize methyl groups from one-carbon precursors (*2*), and the relative specific activities of the creatinine and allantoin excreted by these mice after labeled formate injection indicates that the mouse is able to utilize formate quite effectively as a precursor of creatine. The results also indicate that betaine may be used effectively as a precursor of allantoin. The conversion of methyl to a one-carbon fragment which can serve as a precursor of the β-carbon of serine (*3*), and which may be incorporated into purines (*4*), has been demonstrated. Betaine was more effective as a creatine precursor than as a precursor of allantoin. This is in agreement with the observations that the methyl group may be transferred *in toto* (*5*).

The fact that the leukemic mice excreted more labeled creatinine after sodium formate or betaine injection is proof that at least a part of the increased creatine excretion by leukemic mice is the result of an accelerated rate of creatine synthesis (*1*). These results in conjunction with other findings indicate that creatine (*1, 6*) and methyl groups (*7–9*) must play a significant role in white blood cell formation.

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Effect of Excess Dietary DL-Methionine on Liver and Kidney Catalase of Rats[1]

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In a preliminary investigation upon the effect of single amino acids on catalase activity, we have noted an effect of excess DL-methionine on liver and kidney catalase activity. Protein-free diets containing concentrations of 3–5% of glycine, L-cystine, arginine, leucine, tryptophan, and asparagine were without effect on the catalase activity of protein-depleted rats. DL-methionine produced a marked depression of kidney and liver catalase activity, and further data were obtained with this amino acid.

Adult female Wistar rats were placed on a protein-free diet for 2 weeks and then placed on the diets indicated in Table 1. All diets were administered *ad lib.* The protein-free diet and catalase assay method have been described previously (*1*). The toxic effect of 5% DL-methionine at 10% gelatin concentration is prevented by increased levels of dietary protein. This is in accord with previous observations wherein increasing dietary protein reversed the toxicity displayed by methionine toward growth (*2*) and nitrogen balance (*3*).

The depression and elevation of kidney catalase activity with changes in the concentration of dietary

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TABLE 1

METHIONINE EFFECTS ON LIVER AND KIDNEY CATALASE ACTIVITY

Diet after 2 weeks protein-free diet	No. days on diet	Liver catalase activity*	Kidney catalase activity*	Kidney wt (%)
10% Gelatin, 5% methionine	2	222	23	0.73
" " " "	4	173	19	0.87
" " " "	9	87	9	0.95
25% " 1% "	9	418	68	0.80
25% " 3% "	9	276	47	0.78
25% " 5% "	9	386	29	0.83
25% Casein, 5% "	9	485	12	1.07
Control rats on normal diet	—	550 ± 50	36 ± 4	0.72 ± .04
" " " protein-free diet	—	265 ± 30	39 ± 5	0.68 ± .04

* Catalase unit = ml of O_2/sec/100 g body wt from 1 *N* hydrogen peroxide at 0° C.

methionine and protein suggest that there are different effects of methionine toxicity upon the liver and kidney. The present data and previous observations on the effect of excess dietary protein in increasing kidney catalase activity (*4*) indicate that catalase may be involved in some aspect of protein metabolism.

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Comments and Communications

Inactivation of Circulin by Lipase[1]

PETERSON and Reineke showed (*J. Biol. Chem.*, **181**, 95 [1949]) that circulin, a mixture of basic peptides produced by *Bacillus circulans* Q19, loses its antibiotic activity against *Escherichia coli* ATCC 26 when incubated with a lipase preparation that was free of proteolytic activity as tested by the Mett method (F. C. Koch. *Practical Methods of Biochemistry* [1934]). This observation prompted them to suggest that 6-methyloctanoic acid, which circulin is thought to contain in addition to L-threonine, D-Leucine, and L-α, γ-diaminobutyric acid (DABA), is joined to the peptide through threonine by an ester linkage. To avoid premature acceptance of such a view, we wish to stress the fact that some of the assumptions on the basis of which the existence of this linkage was suggested have not yet been proved. For example, Peterson and Reineke believed circulin to be a *cyclic* polypeptide, taking the following into consideration: (1) amino acid composition (threonine, leucine, and DABA seem to be present in a ratio of 1:1:5); (2) the fact that approximately one half of its amino nitrogen is uncombined (the amino nitrogen before hydrolysis was 7.5%; after hydrolysis, 15.8%); (3) the absence of free carboxyl groups, as shown by titration curves and a negative ninhydrin-CO_2 (Van Slyke) test; and (4) evidence that the amino groups of DABA were the only free amino groups in circulin. The fourth line of evidence is subject to some question and is being reinvestigated. It was based on the observation that the 2,4-dinitrophenyl (DNP) derivative of circulin, when hydrolyzed with HCl, apparently yielded no other products than DABA, α-amino-γ-(2,4-dinitroanilino)-butyric acid, threonine, and leucine, as observed by paper chromatography. However, since DNP derivatives of mono-amino acids do not react with ninhydrin, which was used to indicate the position of the various components on the chromatogram, and are visible only because of their yellow color, small quantities of such derivatives may have escaped detection. Moreover, the fact that not all the DABA appeared as its DNP derivative in the chromatogram makes one wonder whether (1) DABA is formed from its DNP derivative by acid hydrolysis, (2) the dinitrophenylation was not carried to completion, or (3) some DABA is combined in the intact molecule. The first hypothesis can be experimentally disproved (R. G. Shepherd, personal communication) and can therefore be excluded as an explanation for the occurrence of free DABA on the chromatogram. The second alternative is not unlikely, inasmuch as the dinitrophenylation was performed in the absence of alcohol, a condition under which the reaction is thought not to go to completion. This, however, does not exclude the third possibility, especially since the data on the combining weight of circulin were inconsistent. If circulin has a combining weight of approximately 300, as claimed, and actually has five free amino groups, its molecular weight should be 1500. However, calculations from the weight of the constituents that circulin is thought to contain indicate a molecular weight

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