distillation through a Hempel-type column packed with glass beads using a reflux ratio of 3:1.

The viscosities were determined with a Fisher-Irany viscometer, and the densities with a bicapillary pycnometer, as described by Lipkin *et al.* (*2*). The water-bath temperature was controlled within 0.05° C.

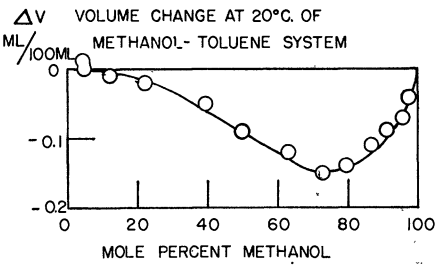

FIG. 2.

The specific heat, heat of mixing, and volume change on mixing of this system at 25° C and 35° C have been studied by Mason and Washburn (*3*), who observed contraction for all compositions and concluded that the associated pure methanol undergoes dissociation when it is dissolved in toluene and that simultaneously there is solvation of the methanol by the toluene.

Similar conclusions were reached by Washburn and Lightbody (*4*) when investigating the volume change on mixing of this system and of other aliphatic alcohols dissolved in benzene or toluene. They also reported contraction throughout the composition range for the methanol-toluene system.

Bushwell, Deitz, and Rodebush (*5*) studied the infrared absorption spectra of methanol and found that the association in concentrated methanol solutions is caused by hydrogen bonding.

Harms (*6*) has discussed the volume changes in this kind of system by assuming that the alcohol exists partly as association polymers of various chain lengths, produced by repeated reaction steps obeying the law of mass action, each reaction step having the same equilibrium constant. According to these views, the observed volume change is the difference between the dilatation caused by depolymerization and the contraction caused by solvation.

The present data show that the viscosity cannot be a function of the volume change alone, but that the reduction in chain length of the association polymers must also contribute to the decided lowering of viscosity when a small amount of methanol is dissolved in toluene.

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Phosphogalactoisomerase[1]

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Caputto and his co-workers (*1*) have shown that the first stages in the fermentation of galactose by the adapted yeast *Saccharomyces fragilis* may be represented by the following equations:

Galactose + ATP ⟶ Galactose-1-phosphate (I)
Galactose-1-phosphate ⟶ Glucose-1-phosphate (II)
Glucose-1-phosphate ⟶ Glucose-6-phosphate (III)

Reaction I is catalyzed by an enzyme, galactokinase, which was separated from the yeast extracts in a partially purified form (*2*). Reaction III is the well-known phosphoglucomutase reaction, which requires diphosphoglucose as a coenzyme (*3*). The enzyme associated with reaction II was not isolated but was detected in the crude yeast extracts by virtue of its requirement for the newly described coenzyme, uridinediphosphoglucose (UDPG). When this coenzyme was added to extracts of the macerated *S. fragilis* cells, an increased rate of formation of glucose-6-phosphate from galactose-1-phosphate was observed (*4*).

In the course of our investigations of the metabolism of galactose in animal tissues we have used the washed cells of *S. marxianis* to remove galactose from tissue extracts and have had occasion to investigate the above reactions with both *S. fragilis* and *S. marxianis*. We have confirmed the essential features of the reaction mechanisms proposed by the Argentine investigators and have extended their observations by the isolation of the enzyme which catalyzes the transformation of galactose-1-phosphate into glucose-1-phosphate. We suggest that this enzyme be named *phosphogalactoisomerase*.

Forty-eight-hr cultures of *S. marxianis*, grown in a yeast extract–galactose media, were harvested and washed with water. The cells were dried *in vacuo* and were then ground to a fine powder. This material was extracted with a phosphate buffer at pH 7.6 and yielded a solution which was rich in the three above-mentioned enzymes. When this extract was acidified to pH 5, a precipitate was formed which was collected and suspended in water. Clarification of this preparation by centrifugation at 8,000 rpm yielded a solution which contained most of the phosphogalactoisomerase but no galactokinase and no phosphoglucomutase.

The enzyme is inactive in the absence of the UDPG coenzyme. However, when this coenzyme is added to a mixture of the enzyme and galactose-1-phosphate, the substrate is converted into a nonreducing phosphorylated hexose which has been identified as glucose-1-phosphate. A typical protocol is shown in Table 1. Since the determinations of glucose-1-phosphate and

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TABLE 1

CONVERSION OF GALACTOSE-1-PHOSPHATE INTO GLUCOSE-1-PHOSPHATE BY PHOSPHOGALACTOISOMERASE AND ITS COENZYME

Enzyme preparation in the incubation mixture (ml)*	Hexose found after acid hydrolysis†			
	0 min		30 min	
	Glucose (mg)	Galactose (mg)	Glucose (mg)	Galactose (mg)
None	0.07	2.30	0.06	2.33
0.2	.09	2.32	.22	2.17
.4	—	2.37	.34	2.03
.6	.03	2.36	.64	1.78
0.8	.08	2.36	.83	1.58
1.0	0.06	2.37	0.93	1.45

* Each flask contained 4 mg of the dipotassium salt of galactose-1-phosphate (equivalent to 2.0 mg of galactose) dissolved in 1 ml of 0.15 *M* phosphate buffer of pH 6.95. To this was added 0.4 ml of a UDPG solution prepared according to Caputto *et al.* (*4*), 0.1 ml of 0.08 *M* magnesium chloride, the quantity of the enzyme solution indicated in column one, and water to make the volume of the mixture to 3 ml. Incubation temperature was 37.5° C.

† The values for galactose include an increment of non-fermentable material derived from the UDPG.

galactose-1-phosphate require the removal of the phosphate radicals by acid hydrolysis and involve a differential fermentation of the glucose and galactose in the reaction products, all data are calculated in terms of the quantities of these two sugars found before and after incubation. The details of the preparation of the enzyme and the analytical procedures used will be reported elsewhere. The data in Table 1 demonstrate that under the experimental conditions used the rate of conversion of galactose-1-phosphate into glucose-1-phosphate is a function of the amount of the enzyme present in the reaction mixture. The mechanism of this reaction is not definitely worked out, and as yet we have not been successful in demonstrating the presence of the enzyme in animal tissues.

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An Elevated Excretion of Creatine Associated with Leukemia in Mice[1]

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In a previous report we have shown a relationship between creatine excretion and the level of circulating leucocytes (*1*). Monkeys recovering from aminopterin treatment exhibited a marked creatinuria, the peak of which corresponded approximately with the point of maximum white blood cell response. Other experiments have indicated a relationship between methyl donors and white blood cell formation (*2, 3*). The present report presents data which show that leukemic mice exhibit a marked creatinuria.

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TABLE 1

CREATINE CONTENT OF URINE AND SKELETAL MUSCLE FROM CONTROL AND LEUKEMIC MICE

	Urinary creatinine (mg/100 g body wt/day)	Urinary creatine (mg/100 g body wt/day)	Muscle creatine (mg/100 g)
Control	1.8	2.6	371
Leukemic	2.1	9.4	361

Mice of the DBA strain, line 2, were used, and leukemia was induced by blood transfer. Approximately 0.1 ml of blood from a leukemic donor was inoculated intraperitoneally into the recipient. We have consistently found that mice so treated develop characteristic blood changes 8 or 9 days following the blood transfer and die in 9–12 days. There are characteristic tissue changes at the site of inoculation. In the first experiment, 4 leukemic mice taken 10 days after blood transfer and 4 control mice were placed in metabolism cages. A single pooled 24-hr urine sample was analyzed for creatine and creatinine (*4*). The animals were then sacrificed, and the creatine content of samples of skeletal muscle was determined. In a second experiment 10 mice were placed in metabolism cages immediately after blood transfer, and creatine and creatinine (*4*) and allantoin (*5*) were determined daily on appropriate urine aliquots. Creatine/creatinine, and allantoin/creatinine ratios were calcu-

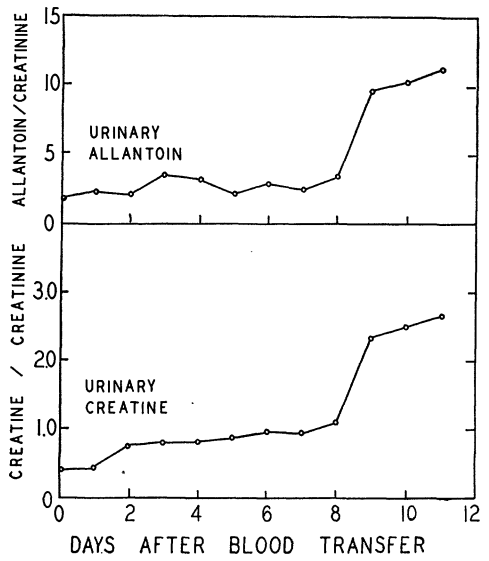

FIG. 1. Daily allantoin/creatinine and creatine/creatinine ratios of urine from leukemic mice.