glycerol portion of the phosphoglyceride molecule, but neither the base nor the fatty acid(4). Moreover, glycine-2-C^{14} labels the base (particularly the serine of phosphatidyl serine and the ethanolamine of phosphatidyl ethanolamine), but does not label to any significant degree the glycerol or the fatty acid portion of the molecule(4). It would thus appear that previous exposures of rats to a cold environment causes a decrease in the subsequent turnover of the fatty acid portion of the phospholipide in respiring liver slices, with no change in the turnover of other portions of the molecule. The finding that, with acetate-1-C^{14} as precursor, cold exposure causes a decrease in the labeling of the fatty acid portion of the phospholipides of rat liver slices, taken in conjunction with the report of Masoro, Cohen and Panagos(7) that under similar conditions there is a decrease in the labeling of the total fatty acid, might suggest that after cold exposure there is some decrease in the ability of the liver slice to metabolize acetate. However, the observations that cold exposure causes no significant decrease in the incorporation of labeled acetate into cholesterol(3,7), or in the oxidation of labeled acetate to CO_2(7) indicates that any change in acetate metabolism must be subsequent to its activation to form acetyl-CoA.

Summary. In rat liver slices respiring in a buffered medium containing suitable radioactive precursors, the acetone-insoluble lipide (phospholipide) is labeled metabolically. Exposure of the animals to an environmental temperature of 4-5°C for the 12 days prior to the experiment caused a decrease in the subsequent labeling of the phospholipide from acetate-1-C^{14}, but no significant change when inorganic P^{32} was the source of the radioactivity. Exposure of the rats to —5°C for the 3 hours immediately prior to the experiment caused a decrease in the labeling of the phospholipide when the radioactivity was derived from acetate-1-C^{14}, but no change when it was derived from glycerol-1-C^{14}, glycine-2-C^{14} or inorganic P^{32}.

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Effects of 6-Mercaptopurine on Respiration, Anaerobic Glycolysis and Succino-Dehydrogenase Activity in Normal and Tumor Tissues.* (22605)

ENRICO MIHICH,† DONALD A. CLARKE, AND FREDERICK S. PHILIPS.

Institute of Pharmacology, University of Milan, Italy, and Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City.

It has been reported that 6-mercaptopurine (6-MP) produces a characteristically prolonged inhibition of the Crocker mouse Sarcoma 180 (S-180). Immediately after treatment with effective doses the majority of tumors fail to "take" when reinoculated into

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† Visiting Research Fellow under a Sloan Stipend for Advanced Study.

normal mice. Moreover, the survival of treated mice is extended and in a significant number tumors disappear completely. In the latter animals the loss of tumors begins as a resorptive process one to 3 weeks after the end of therapy and is completed within another one to 2 weeks(1). Characteristic cytological changes are also seen in treated S-180. There is a predominance of giant, multinuclear cells with both nuclear and cytoplasmic vacuolization. Some of the aberrant cells show irregular, stellate cytoplasmic processes and contain peripherally located, basophilic nuclei. These cytological alterations appear to persist until resorption is complete or growth is resumed.

In spite of the cellular lesion produced by 6-MP, treated S-180 is free of necrosis and is viable by all accepted pathologic criteria. The evidence suggests that the lesion is associated with a persistent metabolic alteration which does not immediately lead to cell-death. To examine this possibility more critically we undertook a study of the respiration, anaerobic glycolysis, and succino-dehydrogenase activity of S-180 and of certain normal tissues of treated mice.

Methods. Mice with S-180 were treated with growth inhibitory doses of 6-MP as previously described(1). Tumors, livers and kidneys were studied 12 hours after the end of treatment. The animals were sacrificed by cervical dislocation and the tissues were placed in iced, buffered Krebs-Ringer solution(2). Slices were prepared free-hand by razor and were introduced into Warburg flasks; they were thin enough so that respiration was not limited by oxygen diffusion. The dry weight of tissue in each flask was about 16 mg in the respiration experiments and about 8 mg in the anaerobic glycolysis experiments. The fluid volume was 3.0 ml in the respiration experiments: 0.2 ml KOH 20% in the central well and 2.8 ml Ringer-phosphate buffer, pH 7.0, in the main well. In the anaerobic glycolysis experiments 2.0 ml of Ringer-bicarbonate buffer with 0.2% glucose (pH 7.1) were used in the main well. In a few experiments neutralized horse serum was used which was prepared as follows: 20 ml of serum were added to 3 ml of HCl, 0.109 N. Evacuation of CO_2 from the acidified serum resulted in a pH of 7.4. Thereafter 2 ml of M/15 phosphate buffer, pH 7.4, were added. The gases used, O_2 and N_2-CO_2 mixture, were flushed through flasks for 10 minutes. The N_2-CO_2 mixture was passed through glowing copper wool before introducing into flasks. Equilibration time was 20 minutes, and readings were made every 10 minutes for 90 minutes following the end of equilibration. Since the values obtained in each reading were uniform throughout the experimental period, Q O_2 and Q $^{N}_{CO_2}$ were calculated on the basis of the total gas displacement. Measurements of succino-dehydrogenase activity were performed on tissue homogenates according to the Schneider-Potter technic(3).

Results. The effects of 6-MP on respiration of S-180 and of liver and kidney were first studied in mice treated intraperitoneally with 50 mg/kg/day for 7 days beginning one day after tumor transplantation. In a few instances the respiration of liver and kidney was also measured in normal animals receiving similar doses of the agent. The results presented in Table I show that treatment decreased the QO_2 of tumor tissue to about 45% of control values though it did not significantly alter QO_2 values of liver and kidney. An analysis of variance revealed that the difference in respiration of S-180 due to treatment was significant beyond the 1% level of probability ($F = 39.11$; p. $0.01 = 11.26$). Although control values varied on different days for unexplained reasons no significant difference was found among experiments ($F = 1.1$; p. $0.05 = 3.44$).

The same order of inhibition of the respiration of S-180 after treatment with 6-MP was observed in manometric experiments during which neutralized horse serum (pH 7.4) was used as a medium in place of buffered Ringer solution (mean values, each of 6 tests: $QO_2 = 7.01 \pm 0.54$ for untreated S-180; $QO_2 = 3.26 \pm 0.80$ for treated S-180).

A few experiments were undertaken in which treatment with the 7 50 mg/kg/day doses of 6-MP was not initiated until the

TABLE I. Respiration of Sarcoma 180 (S-180), Liver and Kidney (Slices).

Animals bearing tumor						Animals not bearing tumor			
Sarcoma 180		Liver		Kidney		Liver		Kidney	
U	T	U	T	U	T	U	T	U	T
6.9	3.0	—	—	—	—	—	—	—	—
6.6	2.5	—	—	—	—	—	—	—	—
6.8	2.9	—	—	—	—	—	—	—	—
7.5	2.9	—	—	—	—	—	—	—	—
6.9	2.1	—	—	—	—	—	—	—	—
6.1	1.6	—	—	—	—	—	—	—	—
*5.0	3.5	—	—	—	—	—	—	—	—
*5.4	2.7	—	—	—	—	—	—	—	—
6.0	1.3	8.7	7.3	—	—	—	—	—	—
5.9	1.4	8.6	9.1	—	—	—	—	—	—
—	—	9.6	11.9	—	—	10.9	11.1	—	—
—	—	9.4	11.4	—	—	12.5	11.1	—	—
—	—	11.2	10.7	—	—	11.5	9.9	—	—
—	—	11.7	11.7	—	—	10.4	9.7	—	—
3.8	3.1	—	—	18.2	23.0	—	—	—	—
4.0	3.2	—	—	23.3	18.8	—	—	—	—
3.2	1.7	—	—	26.5	20.3	—	—	—	—
2.8	1.8	—	—	19.7	25.7	—	—	—	—
—	—	—	—	20.7	20.8	—	—	18.7	20.0
—	—	—	—	25.4	21.1	—	—	20.4	20.3
—	—	—	—	23.7	22.1	—	—	25.5	21.9
—	—	—	—	22.8	29.0	—	—	21.7	22.9
†4.5	1.7	—	—	—	—	—	—	—	—
†4.6	1.0	—	—	—	—	—	—	—	—
†6.5	1.9	—	—	—	—	—	—	—	—
†6.9	2.2	—	—	—	—	—	—	—	—

T = Treated with 6-MP: 50 mg/kg/day for 7 days. U = Controls.
Values indicate O_2 consumption: mm^3/mg dry wt/hr.
* During these experiments anaerobic glycolysis was also measured (Table II).
† During these experiments succinodehydrogenase activity was determined (Table III).
Analysis of variance of tumor data gave the following results: between treated and untreated $F = 23.6$; between experiments $F = 1.0$.

fourth day after implantation of the tumor. In these mice there was a reduction in the QO_2 of S-180 of the same magnitude as that described above (mean values of each of 6 tests: $QO_2 = 5.49 \pm 1.52$ for untreated tumor; $QO_2 = 2.89 \pm 1.17$ for treated tumor). By contrast treatment with a few large doses of 6-MP for a shorter period (250 mg/kg/day for 3 days starting on the 5th day after implantation) did not result in significant reduction in the respiration of S-180.

Values for the anaerobic glycolysis of tumor, liver, and kidney from treated mice are presented in Table II. The $Q^{N}_{CO_2}$ values of the treated tumors are about 35% of the control values whereas those of liver and kidney appear to have been unaltered by treatment. In a few experiments anaerobic glycolysis was determined with horse serum (pH 7.5) as the medium and the result obtained was similar to the findings in Ringer-bicarbonate (mean values, each of 6 tests, not corrected for CO_2 retention: untreated tumor, $Q^{N}_{CO_2} = 11.25 \pm 2.31$; treated tumors, $Q^{N}_{CO_2} = 4.52 \pm 1.22$).

From the above results it is evident that 6-MP induces a significant metabolic depression in S-180. It became of interest to determine whether this effect was due to a generalized lowering of all steps in cellular metabolism or whether it could result from a relatively specific biochemical lesion. For an initial survey of these possibilities the succinic dehydrogenase activities of S-180 and of liver were compared in treated and control mice. From Table III it appears that the succinic dehy-

TABLE II. Anaerobic Glycolysis of Sarcoma 180 (S-180), Liver and Kidney (Slices).

Animals bearing tumor						Animals not bearing tumor			
Sarcoma 180		Liver		Kidney		Liver		Kidney	
U	T	U	T	U	T	U	T	U	T
14.9	7.2	—	—	—	—	—	—	—	—
13.2	6.0	—	—	—	—	—	—	—	—
20.9	5.9	—	—	—	—	—	—	—	—
18.5	5.6	—	—	—	—	—	—	—	—
19.9	6.2	—	—	—	—	—	—	—	—
16.1	6.3	—	—	—	—	—	—	—	—
14.6	5.1	—	—	—	—	—	—	—	—
15.5	5.5	—	—	—	—	—	—	—	—
*27.0	9.1	—	—	—	—	—	—	—	—
*24.8	10.6	—	—	—	—	—	—	—	—
16.9	5.6	2.5	2.2	—	—	—	—	—	—
19.3	4.2	2.6	2.7	—	—	—	—	—	—
—	—	3.1	2.2	—	—	2.9	2.5	—	—
—	—	2.8	2.7	—	—	3.5	3.3	—	—
—	—	3.5	2.8	—	—	2.9	2.0	—	—
—	—	3.4	3.0	—	—	3.1	1.9	—	—
24.6	7.6	—	—	11.0	10.9	—	—	—	—
19.2	7.0	—	—	7.0	11.6	—	—	—	—
—	—	—	—	13.5	11.8	—	—	10.8	11.6
—	—	—	—	12.9	12.3	—	—	11.0	10.9
—	—	—	—	8.3	10.9	—	—	12.8	12.5
—	—	—	—	7.5	11.7	—	—	14.8	11.7

T = Treated with 6-MP: 50 mg/kg/day for 7 days. U = Controls.
Values indicate CO_2 production: mm^3/mg dry wt/hr.
* During this experiment respiration was also measured (Table I).

drogenase activity of S-180 is relatively small. It can also be observed that the treated S-180 had a lower enzymatic activity than the controls; but this difference was small and barely significant at the 5% level ($F = 5.37$; p. $0.05 = 5.32$). No significant difference was found between the activity of treated and untreated liver (mean values, each of 5 tests: 17.68 ± 2.03 for untreated; 20.05 ± 3.27 for treated).

TABLE III. Succino-Dehydrogenase Activity of Sarcoma 180 Homogenates.

Untreated S-180		Treated S-180	
1.2	1.2	1.6	1.5
2.0	1.9	1.9	1.7
1.8	1.7	1.6	1.5
2.0	1.8	1.5	1.7
1.9	1.8	1.6	1.7
1.6	1.6	1.4	1.3
1.7	1.6	1.3	1.3
*1.6	1.8	1.7	1.5
*1.9	1.9	1.6	1.7

$F = 5.37$

Treatment with 6-Mercaptopurine: 50 mg/kg/day for 7 days.
Values indicate O_2 consumption: mm^3/mg homogenate/hr.
* During these experiments respiration of slices of the same tumor was also measured (Table I).

Discussion. The above findings have revealed that the respiration and anaerobic glycolysis of S-180 is reduced by treatment *in vivo* with 6-MP. This metabolic change gains significance when considered in relation to the cytological alteration and inhibition of growth produced by the same treatment. It may be asked whether the observed disturbances in metabolism are the more primary effects of 6-MP that in turn result in retarded growth and altered cellular morphology. To gain further insight into this would require detailed study of various enzyme systems. Such an analysis might consider a previous suggestion that 6-MP or a ribosyl derivative might possibly become incorporated into coenzyme-like molecules in lieu of natural purines(5). The "fraudulent" coenzymes so formed could act as inhibitors of metabolism. This possibility is in keeping with recent experiments in which coenzyme A has been shown to antagonize

the inhibitory effect of 6-MP in tissue cultures of S-180 and of embryo skin(4). It has also been found that the agent inhibits the acetylation of sulfonamide in rat liver homogenates (6).

Summary and conclusions. Chronic treatment with tolerated doses of 6-mercaptopurine causes a reduction of 50% or more in the respiration and anaerobic glycolysis of Sarcoma 180 while it does not impair the metabolic functions in liver and kidney. Succinic dehydrogenase activity of Sarcoma 180 is also impaired by treatment with 6-mercaptopurine, but this effect does not seem to be great enough to account fully for the observed reduction in respiration.

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An Evaluation of Thymidine in Treatment of Tropical Sprue. (22606)

CHARLES E. BUTTERWORTH, JR.* AND ENRIQUE PEREZ-SANTIAGO.†
(Introduced by W. H. Crosby.)

Tropical Research Medical Laboratory, San Juan, P.R., and Department of Hematology, Walter Reed Army Institute of Research, Washington, D.C.

Spies *et al.*(1) demonstrated that thymine given orally in large doses has anti-anemic properties for pernicious anemia patients comparable to folic acid. Vilter *et al.*(2) have noted similar results with uracil. Thymine is capable of replacing folic acid in the growth of a folic acid "dependent" strain, *Lactobacillus lactis*, if vit. B_{12} is provided(3). Thymidine has been shown to be effective in replacing vit. B_{12} in the growth of a number of strains of Lactobacilli under conditions in which vit. B_{12} is ordinarily required(4,5). Thymidine also exerts a marked sparing action on the requirement for folinic acid of *Leuconostoc citrovorum*(3). These results have been taken as evidence that vit. B_{12} is involved in the linkage of desoxyribose with thymine to produce thymidine. Folic acid participates in the transfer of single-carbon units such as the steps leading to the methylation of uracil to produce thymine.

Since folic acid and vit. B_{12} are equally efficacious in the treatment of tropical sprue it seemed of interest to determine the effect of thymidine in the treatment of the megaloblastic anemia of this disease. If there is a block only in the chemical pathways leading to the formation of thymidine, the administration of this end product should correct the defect. To our knowledge there have been no published reports on the use of thymidine in sprue. It has been tried in only small amounts (5 mg) in the treatment of pernicious anemia(6).

TABLE I. Effect of Thymidine on Megaloblastic Anemia of Tropical Sprue.

Patient	Dose (mg)	Route	Total dose (mg)	Reticulocytosis
1	50	I.M.	50	None
2	50	"	50	"
3	100	"	100	"
4	200	I.V.	200	"
5	100	I.M.	600	"
6	100	"	1400	"
7	250	"	2500	"

* Major, M. C., United States Army, Tropical Research Medical Laboratory, San Juan, P. R.

† Bayamon District Hospital, Bayamon, Puerto Rico.