and width of the metaphyses, since these are the factors used in routine assay. The results are indicated in Table 2.

TABLE 2

Ration No.	No. Starting Animals	Av Initial Wt	Weekly Wts 1st	2nd	3rd	Av Wt Gained in 3 Weeks
I	30	48.7	55.9	66.1	74.1	25.4
II	16	49.2	55.4	67.4	81.6	32.4
III	16	47.8	56.4	65.2	72.5	24.7
IV	9	51.8	57.2	72.0	81.8	30
V	10	48.4	55.6	63.4	...	...
VI	13	48.0	53.1	62.6	68.2	20.2
VII	32	47.3	57.6	66.8	78.1	30.8
VIII	14	48.7	56.1	65.3	73.8	25.1
IX	16	51.8	58.7	74.8	88.9	37.1
X	16	47.2	53.1	71.2	79.4	32.2

The figures represent several similar series started at slightly different intervals.

(*a*) During the depletion period the following rats died: 1 each on rations III and VII; 10 on ration V; and 5 on ration VI. (*b*) During the assay period the following rats died: 1 each on rations II and VIII; 2 each on rations I, III, IX, and X; 3 on ration IV; and 4 each on rations VI and VII.

All rachitic metaphyses were of desirable width and character.

Line test results:

a) Rats on rations I and VIII produced medium to thin lines.

b) Rats on rations III, IV, and VI produced thin high lines.

c) Rats on rations II, VII, and IX produced uniform medium lines.

d) Rats on ration X produced lines varying from medium to diffuse.

From the data it is evident that rations III, IV, V, and VI are unsuitable for the standard 21-day period. This may be attributed to an excessive intake of desiccated thyroid or iodinated casein. The detrimental effects assert themselves usually within 2–4 weeks, as evidenced by the number of rats that died during the rickets-producing and assay periods and by the high thin line of calcification, which is usually the result of insufficient food consumption.

The addition of 5 grains of desiccated thyroid to the basal ration produced the best growth, as well as the most uniform lines, when compared with the rest of the respective series. Rations VII and I appear about equal in their ability to promote growth; however, the addition of 0.025% iodinated casein appears to aid definitely in calcification response. Each series of rats received Michigan State College homogenized vitamin D milk calculated to supply 4 U.S.P. units vitamin D. Some variation in the actual potency of vitamin D milks might, of course, be expected. A series containing rats fed 0.025% iodinated casein, 0.0125% iodinated casein, as well as plain basal, were fed milk of somewhat lower vitamin D content because the line test responses were generally smaller. Nevertheless, those receiving ration VII showed better responses.

The fact that rats on ration II seem to do better than rats on ration VII is quite interesting since apparently both represent optimum tolerable levels of desiccated thyroid and iodinated casein. The possibility exists that the thyroid extract may differ somewhat in its over-all physiological activity from that of the iodinated protein.

The results with ration VIII containing 0.0125% iodinated casein indicated that this amount of iodinated casein did not affect the animals with regard to rickets production and the line test.

In no case did the average gain in weight in 3 weeks of rats receiving either ration II or VII exceed the respective basal group by more than 7 g. This may be attributed to the fact that the basal ration is deficient in lysine as well as available phosphorus, thus limiting the effect of either the iodinated casein or desiccated thyroid as growth stimulators. When rations IX and X were employed, the growth rate was greatly increased over the corresponding basal fed group. Furthermore, the incidence of respiratory ailments was greatly reduced by the addition of casein to the ration, thereby providing greater economy in animal usage. The growth attained by the end of the first two weeks was sufficient to suggest that only a two-week period would be necessary for the preparation of rats for vitamin D assays. The results, therefore, compare favorably with those obtained with lysine. The line test results, using rations IX and X, further substantiate the effectiveness of iodinated casein in producing more uniform lines.

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Screening of Potential Cancer-inhibiting Agents[1]

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Fildes (*1*) in 1940 stated that modified essential metabolites should be sufficiently closely related to the essential metabolites on which they are based as to fit the same enzyme, but sufficiently different to be devoid of essential metabolic activity.

Since various investigators (*2*) had reported that the concentration of nucleic acids in tumor-bearing animals is greater than in normal animals, it was apparent that adenine and guanine inhibitors might be found which would retard tumor growth, without ad-

[1] The work described in this paper was sponsored by the Atomic Energy Commission.

[2] Atomic Energy Commission Postdoctorate Research Fellow in the Physical Sciences of the National Research Council, 1949–50.

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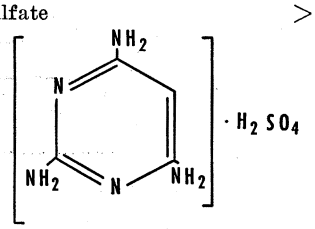
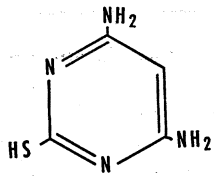
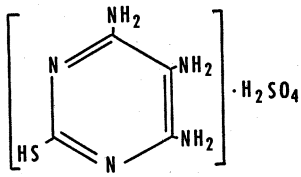
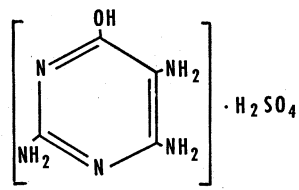
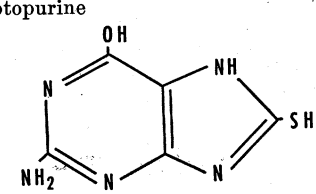
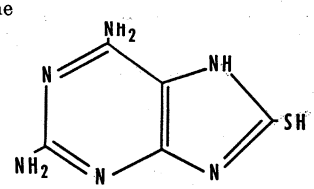
TABLE 1

SURVIVAL TIME OF MICE BEARING MYELOID LEUKEMIA C1498

Ref. No.	Formula and name	MLD/50 mg/20g mouse	Dose mg/20g mouse	No. IP injections weekly	Mean survival time (days)	Mean survival time of controls
1	2,4,6-Triamino pyrimidine sulfate 	> 40	4.0	3	10.8	10.3
2	4,6-Diamino-2-mercaptopyrimidine 	> 40	4.0	3	10.3	10.3
3	4,5,6-Triamino-2-mercaptopyrimidine sulfate 	40	4.0	3	8.0	10.3
4	2,4,5-Triamino-6-hydroxypyrimidine sulfate 	2.5	1.0	3	10.2	10.3
5	2-Amino-6-hydroxy-8-mercaptopurine 	0.5	0.2	3	10.5	9.5
6	2,6-Diamino-8-mercaptopurine 	1.0	0.6	3	5.0	9.5

versely affecting the whole animal. Hitchings (*3*) and Burchenal (*4*) have demonstrated the adenine inhibition of 2,6-diaminopurine, and Roblin (*5*) and Kidder (*6*) showed the antimetabolite action of 8-azaguanine.

In this laboratory we have been working on the synthesis of some thiazolinopurines related to 2,6-diaminopurine[4] and to other purines to be reported later. None of the compounds prepared in this program to date showed any cancer-inhibiting action, but they are being reported in an effort to avoid duplica-

[4] The syntheses of these compounds are being reported elsewhere.

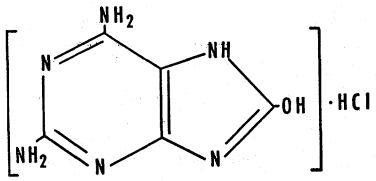
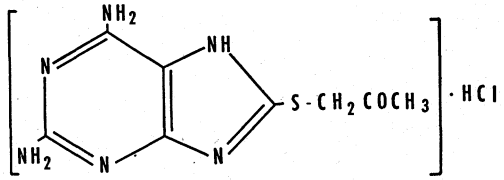
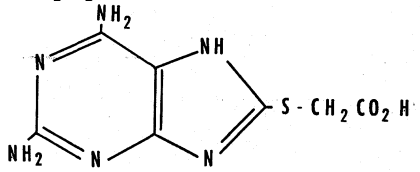
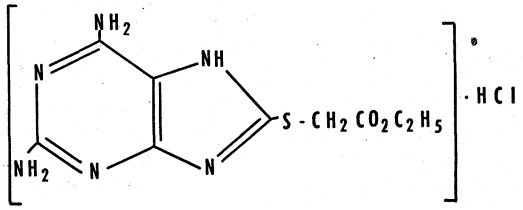
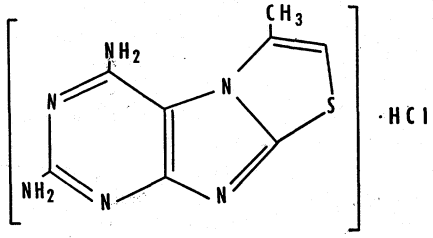
Ref. No.	Formula and name	MLD/50 mg/20g mouse	Dose mg/20g mouse	No. IP injections weekly	Mean survival time (days)	Mean survival time of controls
7	2,6-Diamino-8-hydroxypurine · HCl	5.0	0.4	3	4.0	7.5
						
8	2,6-Diamino-8-acetonylmercaptopurine · HCl	50	20	14	6.0	11.0
						
9	2,6-Diamino-8-carboxymethylmercaptopurine	0.05	0.02	3	9.0	9.5
						
10	2,6-Diamino-8-carbethoxymethylmercaptopurine · HCl	0.5	0.2	3	5.0	9.5
						
11	2,6-Diamino-4′-methyl-[3′,2′-h]-thiazolinopurine · HCl	2.5	1.0	3	9.8	10.3
						

TABLE 2

SURVIVAL TIME OF MICE BEARING MAMMARY TUMORS (Eo771)

5	2-Amino-6-hydroxy-8-mercaptopurine	0.5	0.2	3	8.0	22.5
6	2,6-Diamino-8-mercaptopurine	1.0	0.6	3	10.5	22.5
7	2,6-Diamino-8-hydroxypurine · HCl	5.0	0.4	3	23.0	22.5
8	2,6-Diamino-8-acetonylmercaptopurine · HCl	50	20	14	12.5	21.0
9	2,6-Diamino-8-carboxymethylmercaptopurine	0.05	0.02	3	19.5	22.5
10	2,6-Diamino-8-carbethoxymethylmercaptopurine · HCl	0.5	0.2	3	15.0	22.5

tion by other investigators. Some of the pyrimidines reported were used as intermediates in other syntheses, but they are included here since their antileukemic possibilities had not previously been explored.

The customary screening procedure was carried out using C57 black mice into which had been implanted

either the undifferentiated adenocarcinoma Eo771 or the myeloid leukemia C1498. Dosage of the compounds to be screened was arbitrarily set at 40% of the MLD/50 dose, except in cases where the animals reacted unfavorably to this amount.

Recently Skipper (*7*), working with Ak 4 mouse leukemia, reported that substitution of the 8-position of 2,6-diaminopurine eliminates its antileukemic activity. These results are confirmed by our data. Studies are now in progress with purine derivatives, similar to those in Table 1 (6–11), in the adenine, guanine, and isoguanine series.

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Utilization of C^{14}-labeled Glucose by Cardiac Muscle Treated with a Cardiac Glycoside

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Investigations in several laboratories have shown that cardiac muscle tissue exposed *in vitro* to a cardiac glycoside, or isolated following administration of the drug *in vivo*, respires at a markedly elevated rate (*1*). Because this increase in respiration is restricted to intact tissue and is a function of the concentration of appropriate exogenous substrate, it has been attributed to an increase in the rate of permeation of the substrate into the cell. This paper provides evidence that the phenomenon requires a different explanation.

Ouabain in a final concentration of 5×10^{-7} *M* was added simultaneously with C^{14}-labeled glucose to slices of dog myocardium respiring in substrate-free medium. The $C^{14}O_2$ evolved in the oxidation of the sugar was absorbed by the alkali in the center cup of the respirometer vessel. Table 1 shows that the stimulation of respiration by the cardiac glycoside was not accompanied by a greater uptake of glucose; on the contrary, somewhat less glucose was taken up than in the absence of the drug. However, the rate of glucose oxidation was doubled. According to calculations based on the data in Table 1, the fraction of glucose consumed which was oxidized to carbon dioxide and water rose from 16% to 38%. Comparison of the $Q_{C^{14}O_2}$ and Q_{CO_2}

[1] This work was supported by a grant from the Life Insurance Medical Research Fund. The author wishes to express his thanks to Robert E. Olson for a gift of glucose evenly labeled with carbon 14 and for his interest in the problem, and to Elizabeth White for measuring the radioactivity of the samples.

TABLE 1

EFFECT OF OUABAIN ON THE UTILIZATION OF C^{14}-LABELED GLUCOSE BY SLICES OF DOG VENTRICULAR CARDIAC MUSCLE

Treatment	$-Q_{glucose}$	$Q_{lactate}$	$Q_{keto\ acid}$	$Q_{C^{14}O_2}$	Q_{CO_2}	$-Q_{O_2}$
Control	3.03	1.61	0.07	2.97	7.80	8.33
5×10^{-7} *M* Ouabain	2.69	0.63	0.02	6.08	11.07	11.18

~150 mg of slices (wet wt) per vessel, shaken in 2.85 ml modified Krebs-Ringer-phosphate solution (*4*); O_2 atmosphere, 38° C. Glucose (16.1 micromoles) and ouabain added after 45 min; 2-hr incubation period in glucose. All quantities are expressed as µl ideal gas (N.T.P.) absorbed or evolved /mg dry tissue/hr.

values shows that the acceleration of cardiac respiration by ouabain is quantitatively accounted for by the increase in the rate of glucose oxidation.

Table 1 indicates further that lactic acid production by cardiac slices during aerobic incubation in glucose is greatly reduced by ouabain. The $Q_{lactate}$ declined from 1.61 to 0.63. The change in keto acid production was minor in comparison. Since endogenous lactate formation was nil, one can calculate from the data that the amount of glucose consumed which was converted to lactate decreased from 28% to 11%. And, since the decrease in lactate appearance was not accompanied by synthesis of glycogen,[2] it is also evident that most of the extra glucose oxidized under the influence of ouabain would, in the absence of drug, have been glycolyzed.

Suppression of aerobic glycolysis by ouabain has also been observed in smooth muscle (*2*). In brain cortex, on the other hand, which is the only animal tissue besides heart muscle that has been found to respond to cardiac glycosides with an increase in respiration, these drugs strongly stimulate aerobic glycolysis (*3*). The greater abundance of the Krebs cycle phorase enzymes relative to the glycolytic enzymes in heart muscle as compared to brain cortex may be responsible for the difference in the metabolic response of these two tissues to cardiac glycosides.

It has previously been reported (*4*) that in the presence of pyruvate, which increases the rate of oxygen consumption of cardiac slices to the level attained following addition of a cardiac glycoside in glucose or in lactate, the drug causes no further increase in metabolism. One may infer that in cardiac muscle the cardiac glycosides accelerate the formation of pyruvate from glucose and lactate. Experiments aimed to test this hypothesis are now in progress in this laboratory.

Little can be said at this stage concerning the significance of the present findings with regard to the action of the cardiac glycosides on cardiac function. As has been pointed out before (*4*), it seems unlikely that the stimulation of cardiac energy metabolism by

[2] The glycogen content of the slices declined rapidly during the preliminary incubation and remained uniformly very low (about 20 mg/1,100 g) during the experimental period.