jection of the tolerant rabbits, at maturity, with a mixture of hemopexin and transferrin resulted in production of antibody to hemopexin but not to transferrin. Injection of non-tolerant rabbits with the same mixture resulted in production of antibody to both antigens.

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Depression of Whole Blood Folate Activity by Colchicine.* (30430)

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It is well established that colchicine exerts a beneficial effect in the treatment of gouty arthritis in humans, and is capable of producing mitotic arrest in a wide variety of plant and animal cells, but the manner in which these effects are produced is obscure (1,2). Although gouty arthritis in humans is characterized by increased quantities of uric acid in blood and tissues, it has not been possible to demonstrate any alteration of purine metabolism during colchicine therapy. Colchicine has been classified as a "spindle-poison" in contrast to the "chromosome poisons," such as nitrogen mustard and urethane(2). There has not been, to the best of our knowledge, any explanation of colchicine effect in biochemical terms at the molecular level. Recent studies have shown that small doses of colchicine produce abnormal intestinal absorption of D-xylose without detectable alteration in histologic pattern by routine microscopic examination(4). Histochemical techniques on the other hand have revealed prompt and striking reduction in the activity of several oxidative enzymes and co-factors in the mucosal cells of the small intestine(5). Because of the important role of folic acid in the production of cells by the intestinal epithelium and other active tissues, the following investigations have been carried out to explore possible relationships between colchicine and folic acid metabolism.

Materials and methods. Microbiological assays of folic acid activity were carried out by standard methods utilizing *Lactobacillus casei* (ATCC 7469), and commercially available culture medium based on the formula of Baker *et al*(6). Determinations were performed on whole blood. To release the various folic acid derivatives from their conjugated form in red cells, the following procedure was employed, based on the method of Toennies and Phillips(7). Two ml of heparinized whole blood were mixed with 2 ml of 0.05 *N* ammonium hydroxide and allowed to stand at room temperature for 60 minutes. One-half ml of this hemolyzed solution was diluted to 25 ml with freshly prepared 0.5% potassium ascorbate in .05 *M* phosphate buffer, giving a 1 to 100 dilution, and incubated at 37°C for 90 minutes. Conjugase activity was destroyed by autoclaving for 2½ minutes at 15 p.s.i., after which samples were cooled and filtered through Whatman #1 filter paper. Aliquots of 1, 2 and 4 ml were pipetted into culture tubes, 1 ml of the potassium ascorbate buffer solution was added to each tube and the volume brought to 5 ml with distilled water. Five ml of assay medi-

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um were added. The tubes were autoclaved for 30 minutes at 7 p.s.i., cooled, inoculated and incubated for 18 hours at 37°C. Turbidity readings were made in a spectrophotometer at 650 mμ and quantities were calculated on the basis of a concomitantly prepared growth response curve for synthetic PGA. Similar assays were performed using *S. fecalis* (ATCC #8043) as the test organism and appropriate assay medium. Whole blood hemolysates were prepared and handled exactly as for *L. casei* except that the final dilution was 1:10 for these specimens.

Thirty-two adult white Swiss mice were selected, weighed, and fasted overnight. Colchicine (USP) was administered subcutaneously to 16 of them in doses of 0.24 mg/kg. Three hours later, all animals were sacrificed and blood was collected aseptically in heparinized tubes.

Eight healthy fasting mongrel dogs were given 0.24 mg/kg of colchicine (USP) intravenously. Blood was taken immediately prior to injection and at intervals of 1, 3 and 5 hours afterwards. It is to be emphasized that the doses used in this study are somewhat higher than those ordinarily used in the treatment of human gout, but they produced no visible toxic effects in the treated animals and have been shown previously to produce no morphologic changes in small bowel biopsies.

Normal human adult non-fasting subjects served as donors for an *in vitro* experiment in which 1 ml of heparinized whole blood was placed into each of 4 test tubes. Two tubes contained 0.5 ml of physiological saline solution. The other 2 contained 0.05 mg of colchicine in an equivalent volume of physiological saline solution. One tube of each pair was chilled immediately while the other was allowed to incubate at 37°C for 1 hour. Assays for whole blood *L. casei* activity were carried out at the end of this period for all tubes, as described above.

A standard growth curve was prepared in the usual manner with increments of synthetic folic acid. Identical increments were assayed in the presence of colchicine in a concentration equivalent to 50 times the intravenous dose. Likewise, the standard material was assayed in the presence of 1 ml of dog plasma obtained 1 hour after the animal had received 0.24 mg/kg of colchicine intravenously.

TABLE I. Effect of Colchicine on Whole Blood Folate Activity of Mice by *L. casei* Assay.

	n	WBFA, ng/ml mean ± S.D.
Control	16	1,037 ± 332
3 Hr after colchicine (.24 mg/kg, S.C.)	16	696 ± 309

TABLE II. Whole Blood Folate Activity (ng/ml) of Dogs Before and After Colchicine Administration; Comparison of *L. casei* and *S. fecalis* Values.

			After colchicine (.24 mg/kg I.V.)		
		Untreated	1 hr	3 hr	5 hr
L. casei	1	700	270	363	400
	2	500	288	331	400
	3	300	125	150	175
	4	275	100	150	150
	5	750	425	475	600
	6	300	50		
	7	240	120		
	8	210	60		
		* 409 ± 200	180 ± 125		
S. fecalis	1	10	8	8	8
	2	17	15	21	17
	3	34	28	30	36

* Mean ± S.D.

Results. The results are presented in Tables I, II and III and in Fig. 1. Table III indicates a moderate reduction in folate activity in the colchicine tubes which were cooled in ice water. It is believed that this reduction in folate activity is related to continued metabolic activity of the red cells during the slow cooling period, since this effect was not observed in tubes which were rapidly frozen in dry ice and acetone.

The effect on conjugase activity was tested by adding colchicine to incubation mixtures of either diet and chicken pancreas extract or yeast and normal plasma. As much folate activity was present after incubation with colchicine as without, indicating that colchicine is not a conjugase inhibitor.

Discussion. Statistical analysis indicates that the reduction of folate activity in mice (3 hours), dogs (1 hour), and humans (1 hour), is significant at the 0.1% level. The relative lack of change in the *S. fecalis* assays,

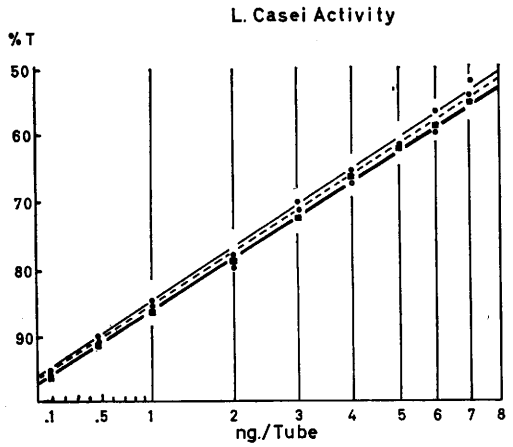

FIG. 1. A standard growth curve for *L. casei* in response to graded increments of synthetic pteroylglutamic acid is shown by the heavy line and black squares. Dashed line and open circles indicate growth response to the same amounts of reference material in presence of colchicine, 50 ng per tube. Light line and closed circles indicate growth response to same amounts of reference material, but in presence of 1 ml per tube of plasma from a dog which had received colchicine intravenously 1 hr earlier (0.24 mg/kg).

(Table II) indicates that the observed effects are not due to a non-specific cytotoxic action, but that the result may be related to some growth characteristic peculiar to *L. casei*. It is pertinent to note in this respect that the growth of *L. casei* reflects the presence of methyltetrahydrofolates and triglutamates, which cannot be utilized for the growth of *S. fecalis*. Both organisms respond to unsubstituted pteroylglutamic acid. Although the relative potency of the various forms of folate for the growth of *L. casei* is not known, our results are compatible with the depression or loss of some form which is more active for *L. casei* than for *S. fecalis*. It is known that the bulk of folate activity of blood is contained in the erythrocytes(8) and that this is principally in the form of methyltetrahydrofolate(9). A review of the known metabolic pathways involving folate indicates that the reduced derivative (tetrahydrofolate) is the active carrier for various single carbon fragments. After the transfer of the carbon fragment tetrahydrofolate is recycled. In the case of thymidylate synthesis, dihydrofolate is formed and must be reduced again to the tetrahydro-derivative. It is therefore possible that colchicine, by depressing some enzymatic step, causes accumulation of one or more heat labile factors, such as THFA and N^{10} formyl THFA(10,11). These could be lost during the routine autoclaving procedure. Another possibility is that colchicine causes conversion of folate to a derivative which is relatively less active in supporting growth of the test organism.

The suggestion has been made(12) that colchicine itself is inactive, requiring conversion to another product within affected cells. This contention is based on the relatively fixed period of delay between drug administration and onset of toxic symptoms, a delay which is not shortened by increasing the dose. Fig. 1 presents results of an experiment designed to test this possibility. It may be seen that neither colchicine itself, nor plasma from a colchicine-treated animal inhibited the normal growth response of *L. casei* to graded increments of synthetic PGA. This growth response curve was observed in spite of the fact that the whole blood folate activity of the treated animal was significantly depressed. This experiment indicates that neither colchicine itself nor plasma which might contain a metabolic derivative acts as a competitive folic acid antagonist. It further supports the view that colchicine is not a non-specific cytotoxic agent, and indicates that the values observed in Tables I, II and III are due to a lowering in the amount of available folate in whole blood samples after colchicine treatment.

The results presented in Table III are thought to be of interest because they demonstrate an effect of colchicine on non-nucleated cells. A prevailing view has been that colchicine does not influence the growth of

TABLE III. Effect of Colchicine on Folate Activity of Human Whole Blood After Incubation for 1 Hour at 37°C *in vitro*.

		L. casei assay, ng/ml, mean ± S.D.			
		Control		Colchicine	
Exp	N	0	1 hr	0	1 hr
1	10	130 ± 31	130 ± 28	86 ± 27	53 ± 19
2	5	80 ± 10	90 ± 6	85 ± 2	35 ± 17

(Note: In Exp 1 the "zero" tubes were prepared by immersion in ice-water; in Exp 2 they were frozen in a mixture of dry ice and acetone.)

non-nucleated cells such as bacteria(13), although there has been divergence of opinion on this point(14). It is established that bacteria have a functional nuclear apparatus, however, so that failure of colchicine to inhibit their growth cannot be correlated with the absence of nuclear material. The mature mammalian erythrocyte, on the other hand, is virtually devoid of nuclear material, so that the data are believed to indicate an effect of colchicine on folate pathways which are not exclusively related to cell division. The role of folate in the metabolism of the mature erythrocyte has not been investigated. However, red cells are capable of several synthetic reactions including formation of glutathione(15) and NAD(16). For the synthesis of NADPH and for the first step of glucose oxidation by the hexose monophosphate shunt pathway erythrocytes are dependent upon glucose-6-phosphate dehydrogenase. Colchicine impairs the activity of this and several other dehydrogenases in the small intestine, liver, kidney and spleen(5) and presumably a similar effect could occur in red cells. Our data do not indicate whether colchicine's effect may be a direct one on the enzymes needed in the recycling process of the folates or an indirect effect brought about by limiting supplies of an essential cofactor such as NADPH.

The important role of folic acid in the transport of single carbon fragments for nucleoprotein synthesis, and the lowering of folate activity by colchicine suggest that the anti-mitotic effect of colchicine may be mediated by a folate-dependent pathway.

The view that colchicine alters folate metabolism is also supported by clinical descriptions of toxicity due to colchicine(17) and desacetyl-methyl colchicine(18). These agents produce alopecia, diarrhea, liver damage, stomatitis, pancytopenia, and bone marrow changes which are similar to those resulting from aminopterin.

Summary. Injection of small doses of colchicine (0.24 mg/kg) produces in mice and dogs a significant decline in whole blood folate activity for *L. casei,* within one to 3 hours. Incubation of normal human whole blood with colchicine *in vitro* for one hour also produces a significant decline in folate activity for the same organism. There was no evidence that colchicine acts as a competitive folate antagonist in the bacterial assay system. It is suggested that colchicine may act by interfering with the action of one or more enzymes which are concerned with the recycling of tetrahydrofolate.

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