are several reasons why the growth of leucocytes in these cultures would not be expected to equal that occurring *in vivo*. Among these are the fact that the cultures were suboptimum as compared to the best culture technique, in containing too many cells per unit volume, in depth of layer over most of the cells, which regulates the O_2 tension, in composition of the medium, and in that no change of medium was made. Furthermore, there is no assurance that a sample of leukemic blood contains the same proportion of undifferentiated cells capable of division as is present in the total blood-forming tissue of the leukemic patient.

The data presented appear to demonstrate that growth of leucocytes from patients with chronic granulocytic or acute lymphocytic leukemias takes place *in vitro*, and that the rate of new cell formation can be estimated by the rate of uptake of P^{32} in the DNA fraction, either *in vivo* or *in vitro*.

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D-Catechol and Antihistaminogenesis

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It has been agreed (*1, 2*) that certain flavonoid compounds have been found capable of the *in vitro* inhibition of an enzyme possessing the capacity of decarboxylating histidine to histamine (*3–5*). Of these, D-catechol was reported as being the most active (*1*). However, conflicting reports (*6–12*) have appeared regarding the usefulness of the flavonoids in the *in vivo* inhibition, as evidenced by experimental sensitization phenomena. Thus, Moss, Beiler, and Martin (*12*) have indicated that D-catechol protected guinea pigs against the anaphylactic shock reaction in contradistinction to the findings of Clark and Mackay (*11*), who confirmed the reported (*7, 9, 10*) negligible effect of the flavonoids. Because of the far-reaching potentialities of an antihistaminogenic compound in clinical allergic diseases, wherein the manifestations are often the result of histamine release, it was felt desirable to repeat, verify, and amplify the findings.

In vitro inhibition. The procedure of Beiler, Brendel, Graff, and Martin (*2*) was followed essentially as described. Reaction mixtures of kidney extract, D-catechol, and L-histidine were prepared. Three control mixtures were simultaneously carried out, each containing only two of the constituents. Isolation of the histamine formed in the reaction was carried out essentially according to the method of McIntire, Roth, and Shaw (*13*) as adapted for chromatographic identification by Urbach (*14, 15*). Histamine, when present in amounts exceeding 2–3 mg, makes its appearance on the filter paper strips as a red band at an R_F value of approximately .56.

In vivo inhibition. Two techniques were utilized to actively sensitize guinea pigs.

Series 1. Seven guinea pigs were sensitized according to the procedure described by Raiman, Later, and Necheles (*8*) as advocated by Moss, Beiler, and Martin (*12*). For seven days prior to sensitization each guinea pig received 2 mg D-catechol daily, given intraperitoneally (i.p.). On the eighth day, in addition to the D-catechol, 0.25 ml horse serum was administered i.p. For 11 subsequent days the animals were each given 2 mg D-catechol i.p. daily. On the twelfth day, the animals were challenged by the intravenous (i.v.) administration of 0.05 ml/100 g body weight of horse serum. Ten control guinea pigs were each sensitized by the i.p. injection of 1 ml of a 1 : 4 dilution of horse serum in physiological saline, but received no D-catechol. On the thirteenth day challenge was accomplished by the i.v. administration of 0.2 ml

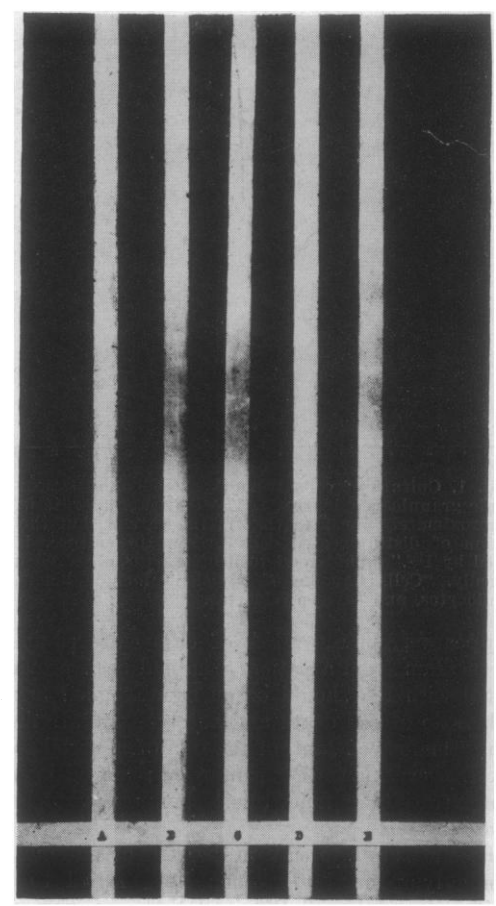

FIG. 1. *A*, kidney extract + D-catechol; *B* and *C*, kidney extract + L-histidine; *D*, kidney extract + D-catechol + L-histidine; *E*, D-catechol + L-histidine.

horse serum (diluted 1:4)/100 g of body weight.

Series 2. Ten guinea pigs were each pretreated by the i.p. injection of 2 mg D-catechol for 4 days. On the fifth day they each received, in addition, 1 ml of a 1 : 10 dilution of horse serum given subcutaneously. D-Catechol was continued for 13 subsequent days. On the fourteenth day, each animal was challenged by the i.v. injection of 1 ml of horse serum. A simultaneous control group was treated identically except for the D-catechol administration.

In vitro inhibition. D-Catechol effectively inhibited the action of the histidine decarboxylase present in the guinea pig kidney extract. The reaction mixture without the D-catechol showed appreciable amounts of liberated histamine. The other controls were negative. The chromatogram is shown in Fig. 1.

In vivo inhibition. Series 1. As Table 1 indicates,

TABLE 1

RESULTS OF ANAPHYLACTIC CHALLENGE OF ACTIVELY SENSITIZED (TO HORSE SERUM) GUINEA PIGS TREATED WITH D-CATECHOL

Guinea pig No.	Series 1		Series 2	
	Control	Treated	Control	Treated
1	++++	++++	++++	++++
2	++++	+++	++++	++++
3	++++	+++	++++	++++
4	+++	++	++++	++++
5	+++	++	++++	++++
6	+++	++	++++	++++
7	+++	++	++++	++++
8	++		++++	++++
9	++		++++	++++
10	++		++++	++++

++++ Died.
+++ Markedly severe symptoms; collapse with eventual recovery.
++ Severe symptoms; marked respiratory distress.

3 of the control group of guinea pigs died in shock, 4 survived markedly severe anaphylactic symptoms, and 3 suffered severe reactions. Of the test group, 1 died in anaphylactic shock and 6 survived. Of the survivors 2 suffered markedly severe and 4 severe symptoms.

Series 2. All control and test animals died in anaphylactic shock.

There seems to be little doubt that D-catechol can inhibit the action of tissue histidine decarboxylase. However, these results confirm those of others (*7, 9–11*) that the flavonoid is without an appreciable *in vivo* action on the enzyme. Since in our hands the sensitization technique of Raiman, Later, and Necheles failed to produce uniformly fatal results in the control group of animals, we considered the results obtained in the treated group as equivocal. For this reason a sensitization procedure was adopted in which an LD_{100} was employed. The treated animals in this group showed no resistance to the challenge. If D-catechol does have an *in vivo* inhibiting action on tissue histidine decarboxylase, the results suggest either that anaphylactic symptoms are produced by some mechanism other than histamine release or that inhibition is not complete and at least a sufficient amount of histamine is formed to account for the symptoms. However, the severity of the reactions seems to indicate that D-catechol plays but a negligible role in preventing anaphylactic shock in the guinea pig, which presumably results from histamine release.

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Counting of Radioactivity in Liquid Samples

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During the recent work using the counting technique proposed by Freedman and Hume (*1*) for liquid samples, two pitfalls were noted when the samples were not counted immediately. In the present study, aluminum cups were carefully coated with Chem-Lac Lacquer 117-2 (Chem-Laq Products, Inc., Cambridge, Mass.) and allowed to dry. The samples counted contained 10^{-3} *M* silver as carrier and 7.5-day Ag^{111} as tracer in 0.8 *M* potassium thiocyanate solution. The surface of the sample was coated with thinned Chem-Lac Lacquer, as described by Freedman and Hume, and allowed to dry. At least 10,000 counts were taken on each sample to cut the statistical error of counting to 1%.

When 1-ml samples were counted in the coated aluminum cups, appreciable plating-out of silver took place within 24 hr, as evidenced by the substantial decrease in activity in Set 1, Table 1. The plating was also visible to the naked eye. After 2 more days, another complication was evidenced by a substantial increase in count, which more than compensated for the decrease due to plating.

Studies of this increase in counting rate were carried out in lacquered glass cups to avoid changes

[1] The authors are indebted to the Atomic Energy Commission for support of this work.