chlorophyll *b*, chlorophyll *a*, and carotin. Absorption data for chlorophylls *a* and *b* are presented in Fig. 1.

Absorption spectra of chloroplast pigments in various physical states and in different solvents are well known. The principal absorption maxima for chlorophylls *a* and *b* in ethyl ether are 430 and 660 mμ, and 455 and 642 mμ, respectively (*2*). The chlorophylls from the mesophyll of *Hymenocallis* were found to have such absorption maxima (Fig. 1) and were unquestionably chlorophylls *a* and *b*. The green pigment isolated from the guard cells of the epidermal tissue has two absorption maxima, at 420–430 mμ and 660–670 mμ, indicating that it is predominantly chlorophyll *a*. The breadth of the absorption band below 475 mμ for the guard cell pigment, and the lack of complete conformity in shape between the density curves for the guard cell pigment and chlorophyll *a*, suggest the possibility that traces of either carotinoids or chlorophyll *b* or both were associated with the green pigment extracted from the guard cells. Although the developed chromatogram of the guard cell pigments showed only one visible band, it should be noted that the quantity of pigment being handled was quite small.

Numerous and varied experiments have been performed using luminous bacteria, *Photobacterium fischeri*, which glow in the presence of oxygen, to test for photosynthesis in guard cells. Each experiment consisted of three groups of culture vessels, test tubes, or van Tieghem cells, all of which contained active bacteria. One set of cultures contained in addition several strips of epidermal tissue; one set contained a small piece of *Elodea, Anacharis canadensis;* and one set was left without any chlorophyllous tissue. The circumambient solution consisted either of the liquid culture in which the bacteria were growing or of a solution of 0.1% $KHCO_3$, to which a high concentration of the bacteria had been added. After the culture vessels were sealed to exclude further access to air, they were placed in a darkroom, and the fluid in them was deoxygenated either by passing a slow stream of nitrogen gas through each or by the organisms themselves. After deoxygenation was completed, as indicated by the cessation of light from the cultures, the test material was exposed to light for various periods of time and then observed as quickly as possible in the dark. Exposures to several intensities of both natural and artificial (Mazda) light were tried. In every experiment the check cultures, containing *Elodea,* were luminous after exposure to light and the cultures without green tissues were not. Evolution of oxygen in the test cultures containing strips of epidermal tissue, as indicated by the bacteria becoming luminous, was not observed. In brief, all tests for photosynthesis in guard cells by this method were negative.

According to Curtis and Clark (*3*), Alvim through the use of the starch test has obtained evidence that photosynthesis occurs in the guard cells of bean plants. The presence of chlorophyll in the guard cells of *Hymenocallis* suggests but does not prove that photosynthesis takes place in them, since chlorophyllous plants are known which do not carry on photosynthesis (*4*). Failure to demonstrate photosynthesis in guard cells through the use of oxygen-sensitive, luminous bacteria does not prove conclusively that the process does not occur in them, for several reasons. In the cultures containing epidermal tissue there was, relatively, a very small number of green cells in comparison with the total number of nongreen cells. Use of oxygen by the latter may have made it impossible to detect any luminosity of the bacteria in the vicinity of the guard cells even with a microscope. Furthermore, submergence of the epidermal tissue plus the rather drastic treatment to which it was subjected in preparation for the tests may have inhibited photosynthesis.

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Growth of Human Leukemic Leucocytes *in Vitro* and *in Vivo* as Measured by Uptake of P^{32} in Desoxyribose Nucleic Acid[1,2]

Edwin E. Osgood, Jonah G. Li,[3] Harold Tivey, Marie L. Duerst, and Arthur J. Seaman

Department of Medicine,
Division of Experimental Medicine,
University of Oregon Medical School, Portland

By determination of uptake rates of radioactive phosphorus (P^{32}) into desoxyribose nucleic acid (DNA), it is possible to obtain a quantitative measure of the rate of formation of human leucocytes in culture (*1*), and by parallel determinations in the cell-donor patient with leukemia, treated with P^{32}, to compare the rate *in vitro* with that of the same population of leukemic cells in the patient. This information is otherwise unobtainable, since leucocyte death is occurring concurrently with cell division, both in the culture and in the patient. The rate of cell formation supplements the information on the rate of cell differentiation, obtainable from total and differential cell counts, and the rate of mitosis obtainable with colchicine. It is the purpose of this paper to present evidence for the initial statement, and to outline briefly the techniques used and the preliminary results.

[1] A preliminary report.

[2] This investigation was supported by grants-in-aid from the Medical Research Foundation of Oregon; the Damon Runyon Cancer Research Fund; the National Cancer Institute, of the National Institutes of Health, USPHS; and the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council. The P^{32} used was supplied by Clinton Laboratories and obtained on allocation from the Isotopes Division, United States Atomic Energy Commission.

[3] Present address: Division of Preventive Medicine, University of California School of Medicine, San Francisco.

TABLE 1

PARTITION OF PHOSPHORUS IN TISSUES OF AN ADULT PATIENT WITH LEUKEMIA TREATED FOR 1 YEAR WITH RADIOACTIVE PHOSPHORUS*

Tissue	All non-DNA phosphorus				DNA phosphorus			
	P^{31}	P^{32}	Specific activity	Plasma specific activity (%)	P^{31}	P^{32}	Specific activity	Plasma specific activity (%)
Spleen	162	5.16	0.0414	141	69.1	1.10	0.0159	54
Lymph nodes	189	5.74	.0303	103	123	1.41	.0115	39
Liver	288	8.90	.0309	105	33.2	0.32	.0097	33
Kidney	182	4.82	.0265	90	35.7	0.33	0.0093	32
Skeletal muscle	191	2.50	.0131	45	97	0	—	—
Cartilage	32	0.59	.0183	62	14	0	—	—
Brain, cortex	237	0.90	0.0038	13	31.5	0	—	—

* Tissues were obtained, before embalming, 1 hr after death, which occurred 3 days after the last dose of 0.033 μc P^{32}/g body wt. The tissues were kept iced until DNA extraction. All values above are mg P^{31} or μc P^{32}/100 g wet tissue. Note that adequate P^{32} is available in the muscle, cartilage, and brain and that, despite readily measurable quantities of DNA P^{31}, no DNA P^{32} uptake was found in those tissues in which cell division does not take place.

Marshak's (*2*) pioneer work on phosphorus turnover in liver nuclei suggested that incorporation of P^{32} in DNA occurs principally during mitosis. This view is supported by Hull and Kirk (*3*) and by others cited (*3*). Independent evidence from this laboratory in support of this hypothesis comes from three sources. First, incubation of isolated desoxyribose nucleic acid at 37° C with labeled inorganic phosphate of 10,000 cpm/ml activity showed no significant exchange in 24 hr. Second, control aliquots removed from cultures of leukemic leucocytes and maintained at 4° C showed no DNA P^{32} uptake over periods of 4 days. Third, post-mortem determinations of DNA P^{31} and P^{32} in the tissues of a patient with leukemia, treated with therapeutic doses of P^{32} for a period of 1 year, failed to show any measurable uptake of P^{32} in DNA of brain, skeletal muscle, or cartilage—tissues considered by most anatomists to undergo no cell division in the adult—whereas uptake of P^{32} was evident in the DNA of tissues in which mitotic division is known to occur. The results of this study are summarized in Table 1.

The basic culture techniques were those previously described by Osgood (*1*) with the following modifications. Leucocytes from patients with leukemia were isolated aseptically from venous blood, using the phytohemagglutinin technique of Li and Osgood (*4*). The culture medium used is shown in Table 2. Culture vessels were Pyrex aspirator bottles, 4-liter size for 1-liter cultures, or of a similar size ratio for smaller cultures. These bottles were autoclaved with cotton plugs in their tops and rubber vaccine-vial caps (size 1A) wired on the tubing outlets. Cultures of 4,000–10,000 cells/mm³ were made by aseptically introducing the cells through the tubing outlet into the medium; after mixing by gentle rotation, the vessels were placed in the 37° C incubator on their sides, with the tubing outlets upward.

TABLE 2

COMPOSITION OF THE CULTURE MEDIUM

Balanced salt solution			
NaCl	8.00 g	$Na_2HPO_4 \cdot 2H_2O$	0.15 g
KCl	0.37	KH_2PO_4	0.03
$CaCl_2$ (anhydrous)	0.17	$MgSO_4 \cdot 7H_2O$	0.07
$MgCl_2 \cdot 6H_2O$	0.21	Dextrose	2.00

Dissolve in the order given and add distilled H_2O to 1 liter. Sterilize by Seitz filtration. Prepare medium by mixing 1 part aseptically collected human serum with 2 parts balanced salt solution and adding 100,000 units/liter of K salt penicillin G. Adjust pH to 7.60 with sterile *N*/1 NaOH or *N*/1 HCl.

After mixing thoroughly, samples were withdrawn daily for total and differential cell counts and pH determinations. The pH was adjusted to approximately 7.6 daily by the use of sterile *N*/1 NaOH, but the medium was not changed. At intervals of 2 days or more, a sufficient quantity of the mixed culture was aseptically withdrawn, by the use of syringes, to yield about ½ billion granulocytes or 1 billion lymphocytes. The cells were harvested by centrifugation, and, after determination of the packed cell volume, DNA was determined by a modification of the Schmidt-Thannhauser (*5*) method, in which the packed cells were dissolved directly in the *M* KOH. The precipitate that resulted from this technique was washed six times with ice-cold 0.5 *N* HCl and 5% trichloracetic acid, redissolved, ashed, and its P content determined by the method of Fiske and SubbaRow. An aliquot of the solution used for the determination of DNA phosphorus was plated out and the radioactivity determined as described by Tivey and Osgood (*6*). Total P^{31} and P^{32} were determined on aliquots of the medium.

When possible, parallel determinations of P^{32} uptake in DNA were made in the patient and in culture. The P^{32} level in the plasma of the patient depends upon the initial dose needed by that particular patient (*7*), and to obtain equivalent radiation effects the P^{32} content of the culture was made equal to the plasma level in the patient, as calculated by the formulas described by Osgood and Tivey (*8*). Daily injections of one tenth or less of the initial dose served to maintain the plasma P^{32} level of the patient essentially constant. Since the dose of P^{32} injected varies from

one patient to another, all final results were made comparable by expressing DNA P^{32} in terms of percentage of specific activity (total P^{32} in $\mu c/100$ ml ÷ total P^{31} in mg/100 ml) of the plasma or of the culture medium.

The results of a culture of cells from a patient with chronic granulocytic leukemia are shown in Fig. 1; those from a patient with acute lymphocytic leukemia in Fig. 2. Data on 5 uptake cultures of cells from patients with chronic granulocytic leukemia are shown in Fig. 3. Obviously, the rate at which P^{32}

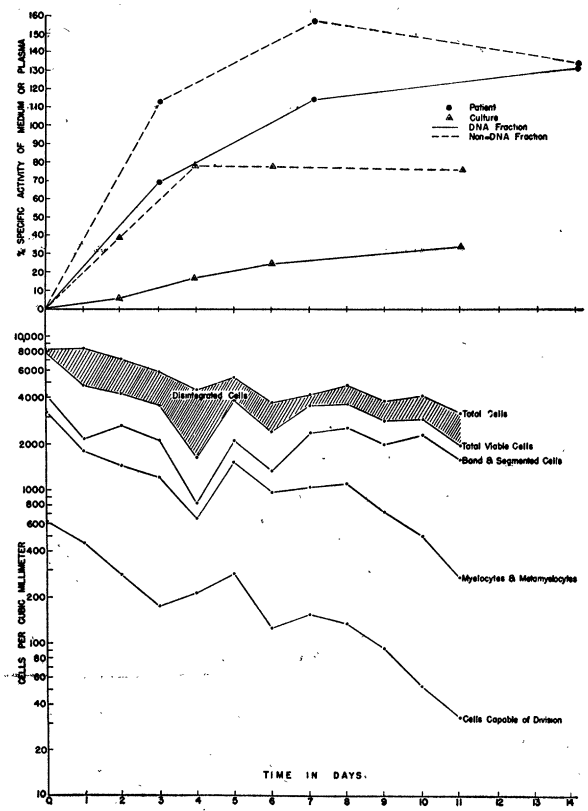

FIG. 1. Culture of cells from the blood of a patient with chronic granulocytic leukemia. The DNA uptake rate *in vitro* is approximately one fifth that in the patient, but the large fraction of distintegrated cells present in culture, if "unlabeled by P^{32}," would tend to decrease the observed uptake markedly. "Cells Capable of Division" include blasts, progranulocytes, and early myelocytes.

disappears from the DNA of cells cultured in P^{32}-free medium from a patient who had been previously treated with P^{32} should be an equally good measure of the rate of cell death and autolysis and, if the cell count remains constant, of the rate of new cell formation. However, since P^{32} cannot be removed instantly from extracellular fluid, this technique cannot be employed *in vivo*.

Three attempts to culture the cells of patients with chronic lymphocytic leukemia have failed to show significant uptake of P^{32} in DNA over periods of time up to 10 days, although uptake of P^{32} in the non-DNA fractions occurred. This result is to be

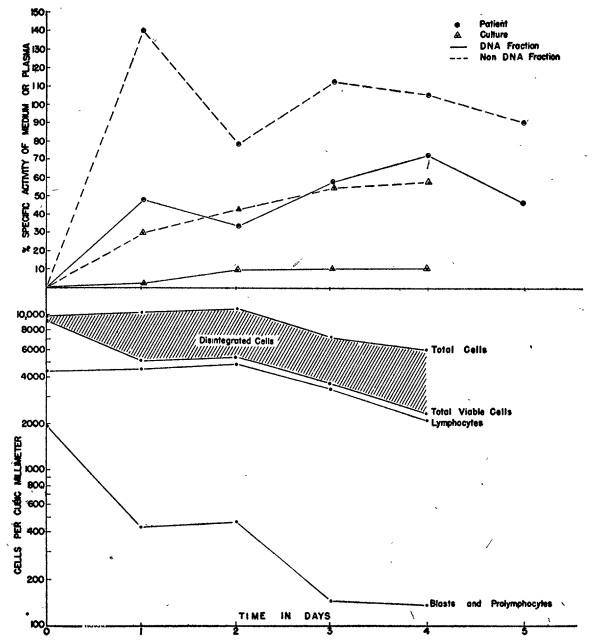

FIG. 2. Culture of cells from a patient with acute lymphocytic leukemia. DNA uptake of P^{32} in culture is small, though significant, and in marked contrast to the negligible uptake observed in cultures of cells from patients with chronic lymphocytic leukemia.

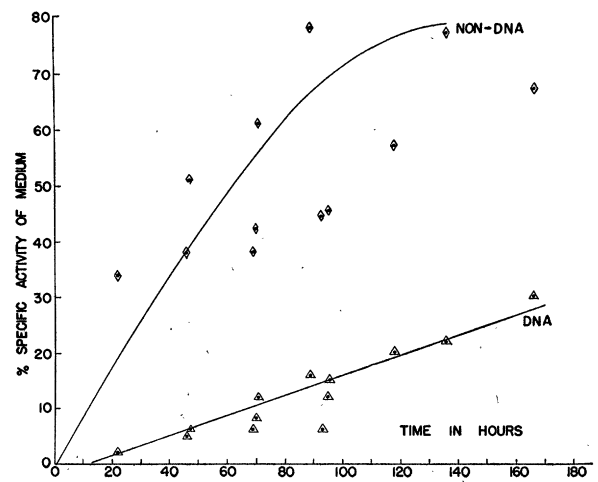

FIG. 3. Results of 5 cultures of cells from patients with chronic granulocytic leukemia. Uptake rates of P^{32} in DNA (indicative of cell division) of approximately 1% of medium specific activity per 10 hr may be noted. Non-DNA P^{32} uptakes are much faster. The sole P^{32} in the medium is introduced as inorganic P. Serum organic compounds totaling approximately 50% of all P present were initially unlabeled. If these latter compounds are also used in new DNA formation, the indicated uptake rate, estimated by DNA P^{32} specific activity ratio to that of the medium, is much lower than that which actually takes place.

expected in view of the uniformly slow rate of new DNA formation observed *in vivo*.

The slope of the uptake curve of P^{32} in DNA in the cultures of cells from patients with chronic granulocytic leukemia indicates new cell formation of at least 20% of that observed in the patient. There

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

Saul Malkiel and Margaret D. Werle

Allergy Research Laboratory, Department of Medicine, Northwestern University Medical School, Chicago, Illinois

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

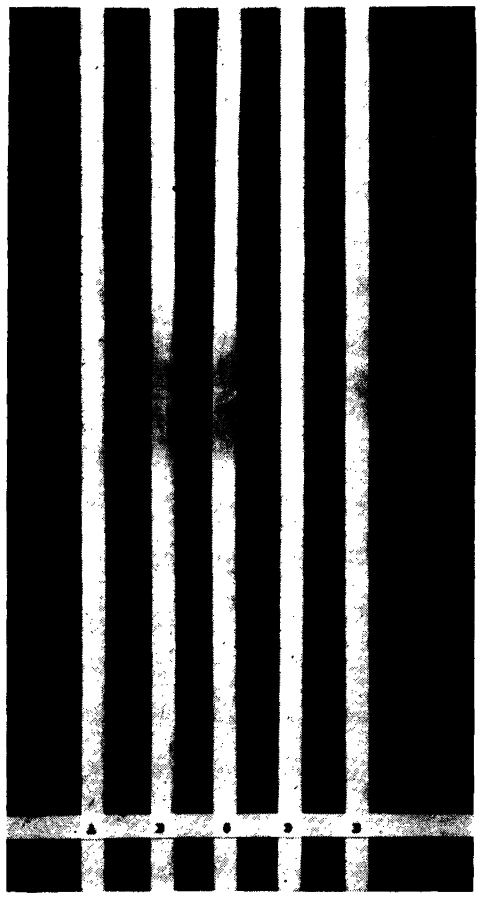

FIG. 1. *A*, kidney extract + D-catechol; *B* and *C*, kidney extract + L-histidine; *D*, kidney extract + D-catechol + L-histidine; *E*, D-catechol + L-histidine.