policy that we established has been adhered to up to the present time, although the Navajo tribe has passed and tried to enforce ordinances against the use of peyote. Administratively, we felt that we had no right to veto the Navajo Tribal Council's action.

There remain on the books of a number of states (procured by the Indian Bureau and by missionaries in earlier times) statutes that declare the ceremonies of the Native American Church (in effect) to be misdemeanors; and there is an occasionally renewed drive at Washington to secure the classification of peyote as a habit-forming and injurious drug. The subject concerns Indians in a dozen or more states; and it is hoped that the communication in SCIENCE will be given attention.

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Measuring Rate of Growth of Leucocytes

RECENTLY, Osgood, Li, Tivey, Duerst, and Seaman (*1*) reported a method for quantitatively measuring the rate of growth of leucocytes in tissue culture, by determining $DNAP^{32}$ (radiophosphorus in desoxyribonucleic acid). In the opinion of the writer, the paper presents no acceptable evidence for the validity of the method. On the contrary, it serves to illustrate a number of errors that must be avoided in this type of work.

Interest in $DNAP^{32}$ has been stimulated by the fact that its rate of formation is so low that it may be dependent upon the rate of formation of new DNA, associated with mitosis. Indeed, advantage has been taken of this fact (*2*) to obtain a qualitative indication of mitotic activity. But, as Hevesy has remarked (*3*), it is of the utmost importance to purify the sample of DNA very carefully, in order to remove phosphorus compounds that may have a much higher specific activity. Many workers (*3–5*) have perfected elaborate methods for isolation of DNA and have presented analyses to characterize their preparations—in other words, to show that DNA was actually isolated and that $DNAP^{32}$ was actually determined.

For isolating DNA, Osgood *et al.* utilized a procedure introduced by Schmidt and Thannhauser (*6*) —alkaline hydrolysis of ribonucleic acid under conditions that do not render DNA acid-soluble. The authors of the Schmidt-Thannhauser method did not specifically study its application to blood leucocytes, nor did they claim that it offered the precision necessary for isolation of $DNAP^{32}$ of any tissue. Furthermore, Osgood *et al.* have omitted the important first part of the method, without any stated reason. Thus their method for determination of $DNAP^{32}$ was essentially a new one, but they presented no analytical data of any sort to justify calling their preparation "DNA." Nevertheless, it is possible to judge the soundness of their procedure, from certain papers that they do not cite. (These studies were concerned with tissues other than blood leucocytes. Although Davidson, Leslie, and White (*7*) applied the Schmidt-Thannhauser method to bone marrow, they apparently did not confirm their results by use of any other method for determining nucleic acids.)

Schneider (*8*) prepared DNA from six different rat organs, according to Schmidt and Thannhauser. To this "DNA" he applied his hot trichloracetic acid extraction procedure, which, as he has shown, separates nucleic acid from non-nucleic acid phosphorus. It was then evident that Schmidt-Thannhauser "DNA phosphorus" was 3–66% too high (compare columns IV-D and V-B of his Table I). Later the Schmidt-Thannhauser method, modified as suggested by Schneider, was applied by the present writer with others (*2*) to $DNAP^{32}$ determination in whole rat liver and hepatomas. It was also applied to nuclei isolated from these tissues. "$DNAP^{32}$" values were greatly reduced, in most cases, if cytoplasm (containing no DNA) was removed. Thus the Schmidt-Thannhauser procedure is not generally applicable to determination of $DNAP^{32}$ in whole tissues. Whether Osgood *et al.* actually isolated $DNAP^{32}$, cannot therefore, be determined from their preliminary report.

That the true rate of $DNAP^{32}$ formation in the cultured leucocytes was actually much lower than the reported rate is suggested by the observations disclosed concerning the degree of vitality possessed by the cells. Generally speaking, the cell counts graphically presented in Figs. 1 and 2 take the form of logarithmic death curves. It must be remembered that it is not possible, with Osgood's techniques, to separate the mitotically active cells, if such are present, from those that are dying. In a paper on marrow cultures (*9*), which Osgood *et al.* cite, Osgood states that his cultures "show degeneration after one to three weeks, although mitoses have been found at thirty-four days. . . ." However, in the tracer experiments reported recently, blood leucocytes, not marrow cells, were cultured, and the culture conditions were stated to be especially unfavorable. Apparently, no mitotic figures were observed.

Finally, Osgood *et al.* claim that by determining $DNAP^{32}$ it is possible "to obtain a quantitative measure of the rate of formation" of new cells. As has been remarked (*5*), the immediate precursor or precursors of DNA phosphorus are unknown. Until they can be identified and the specific activity of phosphorus entering DNA can be determined, there can be no *quantitative* estimate of the rate of formation of DNA, or of new cells, by such an approach.

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IN ANSWER to Cunningham's criticism, we agree that DNA used for specific activity determinations must be carefully purified to remove labeled P^{32} of other compounds of higher specific activity which may be adherent to the precipitated DNA. That this is accomplished by washing the precipitated DNA 6 times with 0.5 *N* HCl in 5% trichloracetic acid is shown in our article by (*a*) the data in Table 1, in which precipitated DNA from brain, skeletal muscle, and cartilage contained readily measurable $DNAP^{31}$ and non-$DNAP^{31}$ and P^{32}, but no detectable $DNAP^{32}$; and (*b*) by the failure to find $DNAP^{32}$ in precipitated DNA from 37° C cultures of leucocytes from patients with chronic lymphocytic leukemia, or in control cultures of granulocytic leukemias kept at 4° C, despite the presence of labeled non-$DNAP^{32}$ in the same cells.

The citation of methods for isolation and purification of DNA used by others (*1–3*) is not pertinent to the criticism of this paper; the method selected for our purposes was the simplest and most satisfactory tried.

The criticism of the failure of Schmidt and Thannhauser to apply their method to leucocytes is irrelevant. Most physicians would agree that a method that they showed to be applicable to thymus tissue would also be suitable for trial on histologically similar leucocytes.

The first part of the Schmidt-Thannhauser (*4*) method was omitted, after parallel analyses on human spleen (from a P^{32}-treated leukemic patient) by the original and modified Schmidt-Thannhauser techniques yielded comparable $DNAP^{31}$ values and specific activities on the precipitated material identical with the chemical and radioactivity assays within the limits of error. The omission was made to simplify the procedure, since only total and DNA phosphorus specific activities were necessary for this paper. Our analyses prior and subsequent to those of the criticized article by the original and modified methods suggest that there is a loss of phosphorus measured as DNA in the extensive pretreatment procedure, but specific activities remain unaltered. The modified method was further checked by quantitative recovery of $DNAP^{31}$ from nucleoprotein (prepared by the method of Mirsky and Pollister [*5*]) that was added to solutions containing serum, albumin, erythrocytes, or even egg yolk. Thus this simple modification, proved not to affect DNA specific activity, does not constitute a "new method." These results are to be published elsewhere.

Cunningham is correct in stating that we present no analytical data to prove our preparation DNA. We followed the custom observed by Cunningham, in his paper quoted above and his references (*5*), (*6*), and (*8*), in letting the chemical procedure describe the product. In the course of these investigations, DNA/10^{12} leucocytes was determined for 11 patients with granulocytic leukemia, and 15 patients with lymphocytic leukemia (48 and 52 determinations, respectively). These values were found to be normally distributed within the same distribution, with a mean value of approximately 600 mg/10^{12} cells ($S \sim 60$). Occasional determinations showed sperm DNA to be about one half this value. These data are not statistically different from the report of Davidson, Leslie, and White (*6*) for DNAP of human leukemic leucocytes of 699 mg/10^{12} cells (S.E. 194).

Cunningham's reference to the earlier paper of Davidson, Leslie, and White (*7*) seems irrelevant, unless he wishes to take exception to their work also. Their determinations were also based on precipitated DNA, and not calculated by difference as originally specified by Schmidt and Thannhauser.

Cunningham's reference to Table I in the paper by Schneider (*8*) shows only that DNA values calculated "by difference" (Column IV-D) are higher than those obtained directly upon the precipitated nucleic acid. This observation was also made in the preliminary studies done in our laboratory, and for that reason all calculations, as stated in our paper, are based on precipitated DNAP, not DNAP calculated "by difference."

Our critic's reference to his own work (*9*) on rat liver DNA seems scarcely comparable to DNA determinations on human leucocytes when one considers (*a*) the difference in species; (*b*) the difference in tissue composition—i.e., liver has not only liver cord cells, but ducts, vessels, and capsule as well; and (*c*) the apparently greater cytoplasmic volume of the rat liver cell in comparison with that of the human leucocyte. His observation that $DNAP^{32}$ values of isolated nuclei were "greatly reduced" over those of whole liver cells is open to interpretations other than the one he gives. Inspection of the bottom rows of his Tables 1 and 2 ($DNAP^{32}$/P) will show the following: (Comparison of specific activities, not P^{32} alone, is here essential.) (*a*) the greatest decrease in $DNAP^{32}$/P is noted in his "control and 3′-MeDAB" values; (*b*) these "control and 3′-MeDAB" values are associated with the lowest $DNAP^{32}$ values; (*c*) the "tumor" groups are associated with the highest P^{32} values; (*d*) the $DNAP^{32}$/P values for these "tumor" groups are for isolated nuclei, 111%, 73% and 102% of the whole liver values at 3 hr, 6 hr, and 6 days, respectively. Unless special precautions (not mentioned) are taken to remove statistical uncertainty of radioactivity determinations associated with low total P^{32} values, one would tend to be much more confident that the relative specific activities of the "tumor" group, which do not appreciably decrease, are much more reliable than those of the low-activity "control" or "3′-MeDAB" groups. His values in Table 2 are modified by his correction for loss of nuclei in separation: ("Judging from desoxyribonucleic acid determinations, 70–80 per cent of the nuclear material of the various whole tissues was recovered in the isolated nuclei, and the values shown in Table 2 were corrected

accordingly.") In view of these factors, and because of the failure to give standard errors, individual data, or other methods of assessing the confidence limits of either Table 1 or Table 2, it is difficult to evaluate the significance of his expression "greatly reduced." These data scarcely provide evidence for the sweeping assertion "Thus the Schmidt-Thannhauser procedure is not generally applicable to determination of $DNAP^{32}$ in whole tissues."

It is true that peripheral blood was cultured in our experiments, but, as any physician knows, there are immature cells capable of division in the peripheral blood, identical with those in the marrow, of patients with leukemic leukemias. This fact is readily apparent from inspection of Figs. 1 and 2 of our paper. The data shown are based on 500-cell differential counts; when necessary, additional cells were counted to enumerate at least 50 cells "capable of division." Segmented neutrophils were present at 11 days in the cultures illustrated, whereas the average time of disappearance of segmented neutrophils from cultures of *normal* blood was 60 hr (*10*). This is further evidence that cell division and differentiation were occurring in these cultures of *leukemic* blood. Mitoses constituted about 0.5% of cells capable of division and were present in all smears of granulocytic leukemia cultures, but no statistically unassailable quantitative estimation was made of the mitotic index. No mitoses were observed in the cultures of cells from chronic lymphocytic leukemias. That cultures of cells from leukemic blood by our methods show mitoses has been confirmed by Gunz (*11*) and many others (*12*).

It is true that cell counts decrease over the period cultured. Particular attention is invited to the fact that $DNAP^{32}$ uptake drops off as the "cells capable of division" fall below 100/mm^3 for granulocytes, or 400/mm^3 for lymphocytes (Figs. 1 and 2). No apologies are made for suboptimum cultural conditions; these were sacrificed to insure enough cells for analysis of comparable volumes of cells in patient and culture, capable of yielding unequivocal chemical analyses, without the use of carrier compounds. The presence of dead and disintegrated cells in these cultures leads to underestimation, not overestimation, of the relative rates of DNA-uptake of P^{32}.

It is quite true that the immediate precursors of DNA are unknown, but it is obvious that inorganic radioactive phosphorus introduced into culture medium or the patient's plasma is the primary precursor of labeled $DNAP^{32}$. The use of total medium or plasma phosphorus specific activity as an interim quantitative guide would make our estimates err on the conservative side. The phenomenon of saturation of $DNAP^{32}$ specific activity equivalent to the specific activity of the patient's plasma, seen in Fig. 1 (and seen repeatedly in other patients with chronic granulocytic leukemia [*13*]), strongly suggests complete turnover of $DNAP^{32}$, especially as later studies have shown that the maximum specific activities of leucocyte acid-soluble and lipid fractions are comparable to these DNA saturation values.

Careful reading of our article will disclose that "quantitative" claims are made only in the sense of relative rates between the *in vitro* and *in vivo* human experiments, and for differences in uptake rates between cells of the lymphocytic series from patients with acute and chronic leukemias, and between cells of the granulocytic series and cells of the lymphocytic series. A *complete* quotation of our original sentence by Cunningham would have made this clear to the reader of his criticism. The desirability of reducing this type of data to a series of chemical steps, the reaction rates of which could be predicted by appropriate differential equations, is not questioned, but both limitations of chemical knowledge and biological variation prevent us (and Cunningham) from such accomplishments at present. Such limitations, however, do not prevent Cunningham *et al.* (*9*) from stating at the conclusion of their cited article, in which mitotic rates are also not given, "Therefore the degree of incorporation of the isotope in desoxyribonucleic acid could be correlated roughly with the degree of mitotic activity."

In conclusion, may we say that the purpose of our paper in SCIENCE was to present conclusive evidence that growth was occurring in cultures containing up to 10 g of human leucocytes, to describe simple modifications of existing methods, and to present further evidence that $DNAP^{32}$ uptake is a measure of the relative rates of new cell formation. The criticisms raised appear to us to originate in misinterpretation of the original article and in debatable interpretation of references, and they in no way invalidate the statements in the original article.

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