At the end of the collection period, the solution contained 15 to 20 lit of CO_2 as Na_2CO_3, which was precipitated as $CaCO_3$ by the addition of $CaCl_2$. The precipitated calcium carbonate was separated by decantation and filtration, was dried, and was placed in sealed containers. By drawing the air through two collecting bottles in series, the efficiency of collection of CO_2 from the air was found to be approximately 80 percent.

The counting technique employed was that developed by Suess (*3*), in which the carbon-containing material is converted first to carbon dioxide and then to purified acetylene. The acetylene is used as the counting gas in a well-shielded proportional counter with an anticoincidence arrangement for reducing the cosmic-ray background. It was found that excellent stability and good repetitive accuracy could be obtained over periods of a year or more.

We were fortunate (*4*) in obtaining small quantities of strontium carbonate prepared from fossil carbon (lignite coal) and from contemporary carbon which had been measured previously (*5*). These samples were converted to acetylene and served as standards for all of our measurements. For each of the experimental values reported here, the counting rate of the atmospheric carbon was compared with two measurements on the contemporary carbon standard and with two measurements on the fossil carbon standard. One standard measurement was made within 3 days before, and the other within 3 days after, the sample count. In some cases, the reported values were derived from more than one measurement, and in other instances two completely separate samples were prepared from the same atmospheric carbon. All of the errors shown were computed from the total number of counts and are expressed as the 9/10 error in this quantity.

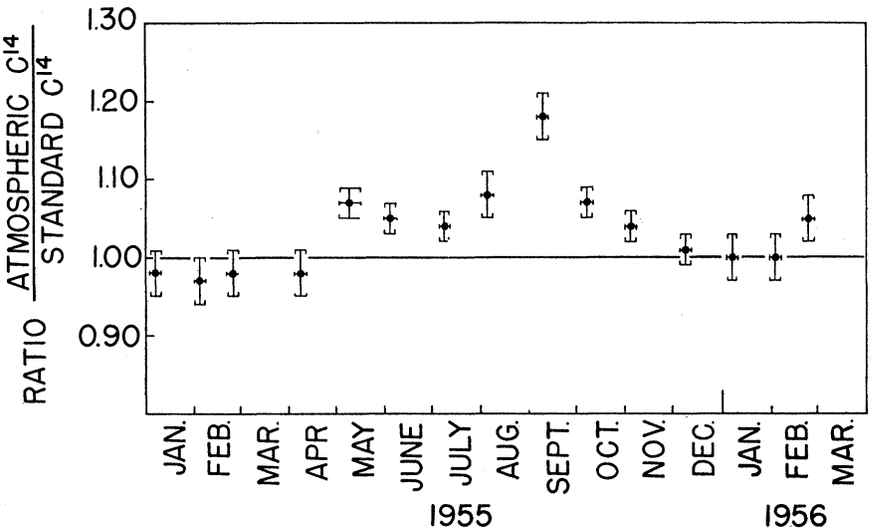

Fig. 2. C^{14} content of atmospheric CO_2 samples collected at Washington, D.C.

In order to test the reproducibility of the entire process, three simultaneous atmospheric carbon samples were collected and separately processed. The counting rates obtained were well within the expected statistical error.

By means of the afore-described techniques, four 1-week samples collected during the period from October to December 1952 in French Morocco and four similar collections in Alaska gave average values for the sample/standard ratio of 0.97 ± 0.01. Four samples collected at Washington, D.C., during this same period gave an average of 0.95 ± 0.01, while three collections made in the Hawaiian Islands and three in the Philippine Islands gave average sample/standard ratios of 1.00 ± 0.02. In no case did the C^{14} content of the atmospheric sample exceed that of the standard. The lower values (particularly at Washington, D.C.) are believed to reflect the dilution effect of the burning of fossil fuels.

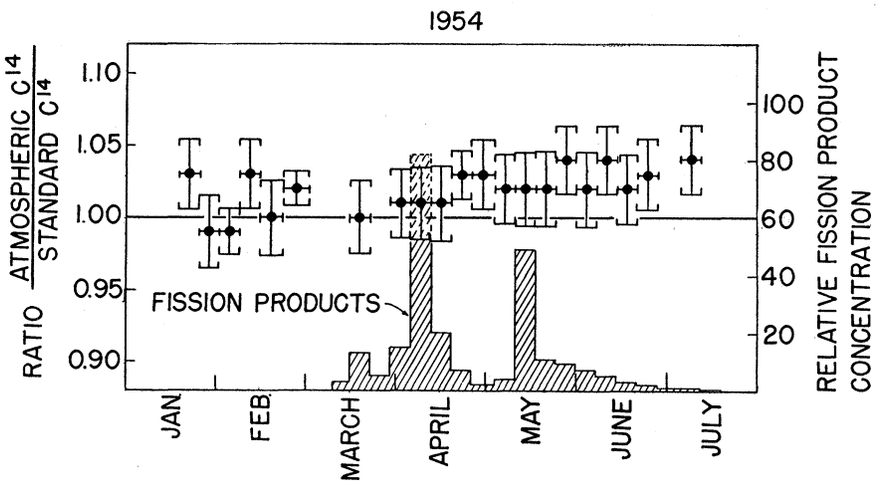

Fig. 1. C^{14} content of atmospheric CO_2 samples collected at Subic Bay, Philippine Islands.

We were able to obtain CO_2 collections from the Naval station at Subic Bay in the Philippine Islands during the thermonuclear tests of 1954. They are of interest, since it was possible to measure simultaneously the ground-level concentration of fission products. The direction of the winds below 20,000 feet from the Pacific proving grounds was such that the observed radioactivity should have been the result of low-altitude fission debris. The counting rates of atmospheric carbon, referred to standard carbon, for CO_2 samples that were collected from January to July of 1954 are illustrated in Fig. 1, along with the relative fission-product concentration for the same period. It is clear that the concentration of C^{14} did not increase as markedly as did that of the fission products. However, it does appear that it was somewhat higher after the tests than at the time of the 1952 collections. It is possible that most of the C^{14} which was formed was entrained in the hot gases of the fireball and injected into the stratosphere so that relatively little was present in the ground-level cloud.

In Fig. 2 are shown the relative atmospheric/standard carbon counting rates from collections made in Washington, D.C., from January 1955 to February 1956. Samples collected in the months of May through November 1955 were significantly higher than the standard in C^{14} content and, in one instance, as high as +18 percent. The fact that the concentration was at a minimum during the colder months may indicate a seasonal decrease, resulting from reduced plant transpiration and the burning of fossil fuels. Almost all of the measurements gave higher values than those of October–December 1952.

Previous measurements of atmospheric C^{14} by Kulp (*6*) indicated no significant deviation from contemporary wood for

12 samples collected under a variety of conditions. Rafter (7), on the other hand, found that four CO_2 samples collected in New Zealand in 1954 and 1955 had higher concentrations of C^{14} than had contemporary wood (+4.7 percent for one sample). Our collections at Washington, D.C., during the summer of 1955 gave values for the C^{14} content of atmospheric CO_2 appreciably higher than those previously reported. It seems difficult to account for these high values on the basis of isotopic fractionation, and therefore the increase in the C^{14} content of atmospheric CO_2 from 1952 to 1956 is probably the result of the addition of radiocarbon from thermonuclear sources. The delayed appearance of the C^{14} increase at ground level may indicate a stratospheric reservoir of this isotope.

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References and Notes

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4. We wish to thank M. Rubin, for supplying the standard samples; L. B. Lockhart, Jr., H. Friedman, and R. A. Baus, for helpful discussions; Paul Gustafson, who assisted in the preparation of some of the samples; and the Naval personnel who collected the CO_2 samples.
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3 March 1957

Melanin Mobilization in Pigment Cells of the Mouse

Many lower vertebrates possess the ability to change color rapidly by effecting redistribution of pigment in the chromophores (*1*) of the skin. The pigment cells have been shown to be quite sensitive to a number of chemical and physical agents (*2*). Epinephrine causes a rather rapid migration of the cytoplasmic melanin granules to the center of the melanophore. Fig. 1*A* shows a melanophore on a fragment of fish fin in Holtfreter's solution. After the saline is replaced with 0.5 m*M* epinephrine in Holtfreter's solution, the pigment mass assumes the configuration of Fig. 1*B* within 30 seconds. If the epinephrine is removed, the pigment is redistributed throughout the cell.

It has been generally assumed that the ability to mobilize melanin is restricted to the pigment cells of only those animals that are capable of effecting rapid

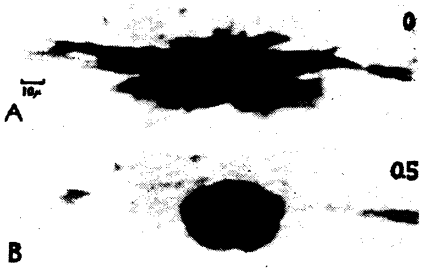

Fig. 1. (*A*) A melanophore from the caudal fin of the fish *Xiphophorus helleri* in Holtfreter's solution. (*B*) The same cell shown in *A* exposed to 0.5 m*M* epinephrine.

changes of skin color. Although no functional melanophores are found in mammals, the mammalian melanocyte does occur, and it is quite similar to the melanocytes of lower vertebrates, especially to those found in pigmented tumors (*3*). Since in earlier studies we had observed pigment mobilization in the tumor melanocytes of *Xiphophorin* fish melanomas (this has also been reported by Greenberg *et al.*, *4*), we suspected that such a phenomenon might also occur in the pigment cells of mammalian melanomas.

The mammalian melanocytes that we first studied were those found in tissue cultures of the Cloudman mouse melanoma (*5*). Melanocytes were abundant and rather easily distinguished from the larger macrophages and pigmentless cells. The nuclei of melanocytes were small and contained only one or two nucleoli, whereas other cell types had large, multinucleolated nuclei. Furthermore, melanocyte behavior, as observed in time-lapse motion pictures, was quite characteristic. Cell shape was ignored, since it tended to be variable.

Tissue cultures of the mouse melanoma were made according to the standard roller tube method (*6*). After about 1 week of culturing in horse serum medium (*7*), the cover-slip cultures were incorporated into a perfusion chamber (*8*). The perfusion medium used throughout the test was that in which the cultures had been grown. Since cytological changes in rounded cells are difficult to observe, the culture outgrowths were searched for flattened melanocytes of the type shown in Fig. 2*A*. A record of the subsequent testing was made with cinephotomicrographic time-lapse equipment (*9*). Color film was used so that the brown melanin granules could be distinguished from other cytoplasmic granulation, especially spherical mitochondria.

Time-lapse motion pictures made preliminary to treatment established that normal activities consisted typically of rapid membrane undulation, occasional pynocytosis (cell drinking), and considerable erratic motion of the melanin granules and mitochondria in the body of the cell. Such a cell in untreated medium is shown in Fig. 2*A*.

When the chamber medium was replaced with culture medium containing 0.5 m*M* epinephrine, normal membrane action was immediately suspended, the motion of the cytoplasmic granulation ceased, and all cell movement appeared to be frozen. Melanin granules clumped together, and the pigmented mass in the cytoplasm slowly began to contract. Some of the spherical mitochondria remained in the clear cytoplasmic regions (Fig. 2*B*), but their motion was halted by the treatment.

After the pigmented mass reached what appeared to be a maximum contraction (Fig. 2*B*), the chamber medium was replaced with untreated culture medium. Immediately the cell membrane

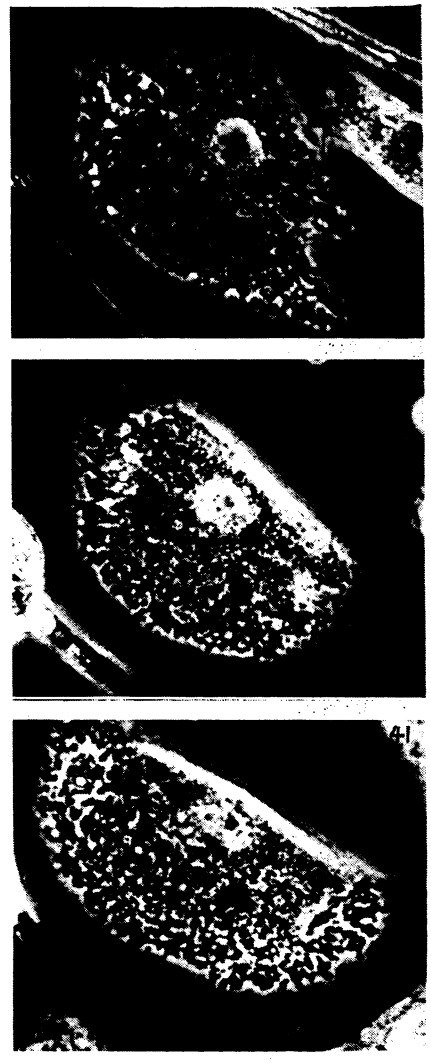

Fig. 2. (*A*) A melanocyte from the Cloudman mouse melanoma in tissue culture. (*B*) The same cell exposed to 0.5 m*M* epinephrine. (*C*) The cell after the removal of the epinephrine. The number in the upper right of each photograph indicates the time interval in minutes from the preceding picture.