an additional 2.7×10^{19} ev. The total incorporations obtained were 0.16 μc for the control and 1.11 μc for the γ-irradiated sample; thus, the incorporation was increased by a factor of 6.9. This factor is similar to that found in the previous experiment, and the total activities incorporated in the two experiments are, therefore, proportional to the HT concentrations.

These experiments indicate that the γ-radiation does, as expected, increase the rate of tritium labeling. However, the factor of increased labeling (6.8 or 6.9) is much lower than the increase in energy delivered to the system (57 in one case, 207 in the other). Consequently, it appears that the increased labeling is obtained only at the expense of molecular destruction—that is, the γ-radiation increases the ratio of destruction to labeling. It is thus inferior to exposure to higher tritium specific activities as a means of increasing the amount of labeling for a given exposure time.

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Bilirubin Inhibition of Heme Biosynthesis

Abstract. The conversion of protoporphyrin and iron to heme is catalyzed by a soluble enzyme prepared from rat liver. This reaction is inhibited by bilirubin, and initial kinetic studies suggest that the inhibition is due in part to a competition between protoporphyrin and the bile pigment. Implications of this finding in hyperbilirubinemia are mentioned.

Hyperbilirubinemia, a condition in which the blood concentration of unconjugated (indirect) bilirubin is markedly increased, is a frequent clinical problem in the newborn infant. In these cases hyperbilirubinemia may be accompanied by serious brain injury. Since brain tissues are very sensitive to even mild states of anoxia, any interference with their ability to carry out biological oxidation might result in cellular damage. Bilirubin has already been implicated experimentally in this way through its inhibition of aerobic oxidation and uncoupling of phosphorylation in both brain and liver tissue (*1*). A more specific, but perhaps related, biochemical effect of bilirubin is described in this report (*2*).

In this laboratory we have been studying the mechanism of the reaction by which iron and protoporphyrin combine to form heme (*3*). The reaction is the last step in heme biosynthesis and is catalyzed by a soluble enzyme found in such varied tissues as rat and chick embryo liver, beef heart, chicken erythrocytes, and rat brain. On comparing the structure of one of the substrates, protoporphyrin, with bilirubin it can be seen that they are both tetrapyrroles with identical side chains and that they can exist in spatially similar arrangements. The work of Granick and Gilder (*4*) with *Hemophilus influenzae* has shown that the propionate groups of protoporphyrin attach to the apoenzyme and that the vinyl groups are essential for iron incorporation. This being the case, it is not difficult to visualize how bilirubin could act as a competitive inhibitor of protoporphyrin utilization, since reduction of the γ-methene bridge between the pyrroles bearing the propionate groups would allow restricted rotation but still have little effect on their relative spatial positions, whereas complete removal of the α-methene bridge could so affect the spatial arrangement of the vinyl groups that they would be nonfunctional.

In order to test this hypothesis we used the soluble enzyme preparation obtained from rat-liver mitochondria and both the isotopic and spectrophotometric assays reported previously (*3*). Only one change was made in these procedures—the reaction was stopped after only 20 minutes' incubation to assure that measurements were made at a constant reaction rate. Bilirubin (*5*) was dissolved in 10-percent sodium carbonate, adjusted to *p*H 8 with HCl, and diluted to a concentration of 0.007*M*.

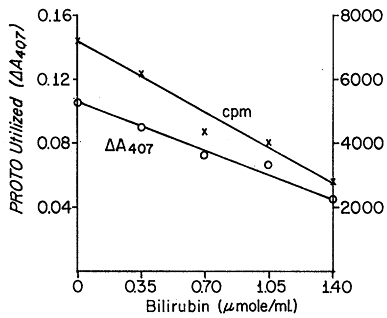

Fig. 1. Effects of bilirubin on heme biosynthesis as measured simultaneously by protoporphyrin utilization and by iron-59 incorporation.

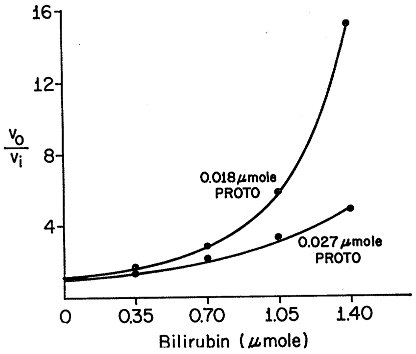

Fig. 2. Kinetics of bilirubin inhibition at different protoporphyrin concentrations.

As is shown in Fig. 1, bilirubin inhibited simultaneously the utilization of protoporphyrin and the uptake of iron-59, the latter to a slightly greater extent. Even though the enzyme was in a crude form, preliminary kinetic studies were carried out in an effort to learn whether bilirubin was inhibiting primarily through competition with protoporphyrin. By varying the concentrations of these two compounds and keeping iron constant, the data plotted in Fig. 2 were obtained. These data suggest competitive inhibition, since the curves for different substrate (protoporphyrin) concentrations are in the proper relation and intersect the ordinate at unity. However, the curves are not straight lines but increase approximately logarithmically with increasing inhibitor concentration. Thus, there may be competitive inhibition with protoprophyrin with a second effect superimposed, perhaps involving the iron directly. As an inhibitor of this reaction bilirubin is quite active, since 50-percent inhibition is obtained with a bilirubin concentration of $10^{-3}M$ and a bilirubin-protoprophyrin molar ratio of 53.

In conclusion, it should be mentioned that bilirubin inhibits this same reaction in rat-brain homogenate. While the data presented briefly here do not explain the varied clinical manifestations of hyperbilirubinemia, they do show that one action of bilirubin in this disease may be inhibition of the biosynthesis of heme, the prosthetic group of numerous enzymes essential for biological oxidation.

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2 February 1959

Stratospheric Fallout of Strontium-89 and Barium-140

Abstract. A series of nuclear test explosions which occurred in the fall of 1958 caused a very large increase of Sr^{89} and Ba^{140} in the stratosphere. The Ba^{140}/Sr^{89} ratio in the stratosphere, and hence in the troposphere, has decreased steadily since then with a half-life of approximately 17 days.

The last nuclear test explosion seems to have occurred sometime in early November 1958, and it appears as if there will be no more test explosions, at least for the time being. Since the fission products remain in the troposphere for only a month or two, the fallout since December or January must have originated almost exclusively from the stratosphere. In other words, we are now dealing with a "pure" stratospheric fallout, and the period of suspension of nuclear tests provides an excellent opportunity to study the mechanism of the stratospheric fallout.

Samples of rain and snow were collected on the roof of the chemistry building of the University of Arkansas, and the Sr^{89} and Ba^{140} contents were determined radiochemically by a method described earlier (*1*).

Three pancake-type counters (Anton 1007TA) surrounded by cosmic ray counters, and placed within an iron shield, were used for the radioactivity measurements. The backgrounds of the counters were 1.7, 2.0, and 1.8 count/min, respectively, in anticoincidence with the surrounding cosmic ray counters. The experimental results obtained are shown in Table 1 and Fig. 1.

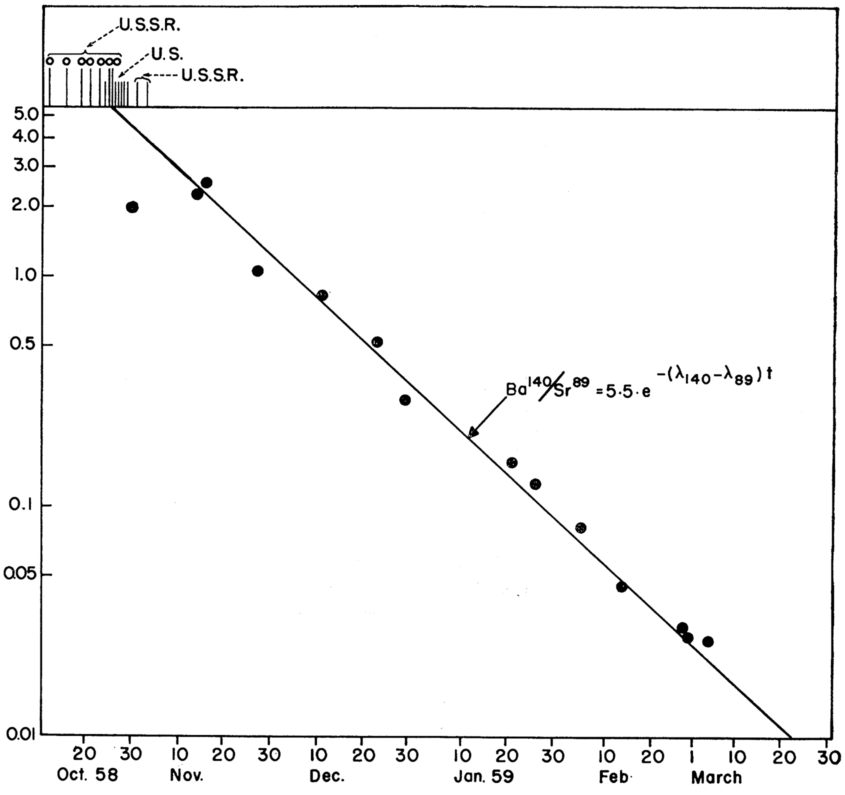

Fig. 1. Variation of the Ba^{140}/Sr^{89} ratio in rain and snow.

Table 1. Barium-140 and strontium-89 in rain and snow at Fayetteville, Ark.

Date	Rainfall (in.)	Ba^{140} (μμc/lit.)	Sr^{89} (μμc/lit.)	Ba^{140}/Sr^{89}
31 Oct. 1958	0.17	2710	1355	2.00
14 Nov. 1958	2.00	569	246	2.24
16 Nov. 1958	1.53	172	67.9	2.54
27 Nov. 1958	0.54 (snow)	325	312	1.04
11 Dec. 1958	0.10 (snow)	114	138	0.83
23 Dec. 1958	very slight	203	392	0.52
30 Dec. 1958	0.50	98.2	352	0.29
21 Jan. 1959	0.50 (snow)	50.3	320	0.157
26 Jan. 1959	0.15	161	1270	0.127
5 Feb. 1959	0.30 (snow)	26.7	325	0.082
14 Feb. 1959	0.65	12.5	277	0.045
27 Feb. 1959	0.50	20.7	691	0.030
28 Feb. 1959	0.30	7.6	284	0.027
4 Mar. 1959	2.00	6.4	250	0.026

The Ba^{140}/Sr^{89} ratios in rain or snow can be expressed by the following empirical equation:

$$[Ba^{140}/Sr^{89}]_R = ke^{-(\lambda_{140}-\lambda_{89})t} \qquad (1)$$

where $[Ba^{140}/Sr^{89}]_R$ is the Ba^{140}/Sr^{89} ratio in rain or snow at the time t of the rainfall, λ_{140} and λ_{89} are the decay constants of Ba^{140} and Sr^{89}, respectively, and k is a constant.

It is interesting to note that the backward extrapolation of the straight line in Fig. 1 to 25 October 1958 gives a value for k of 5.5, which is essentially the same as the Ba^{140}/Sr^{89} ratio in a freshly produced fission product mixture from U^{235} fission. It is known that a series of hydrogen bomb explosions occurred in the arctic during the period between 12 and 25 October 1958.

Equation 1 is a special case of the following general equation which has recently been derived by Kuroda (*2*):

$$\left(\frac{B}{A}\right)_{T,t} = \frac{\dfrac{k_T-k_S}{k_S}\cdot\dfrac{A_{T,0}}{A_{S,0}}}{[e^{(k_T-k_S)t}-1]+\dfrac{k_T-k_S}{k_S}\cdot\dfrac{A_{T,0}}{A_{S,0}}} \times \left[\left(\frac{B}{A}\right)_{T,0}-\left(\frac{B}{A}\right)_{S,0}\right]\times e^{-(\lambda_B-\lambda_A)t} + \left(\frac{B}{A}\right)_{S,0}\times e^{-(\lambda_B-\lambda_A)t} \qquad (2)$$

where $(B/A)_{T,t}$ is the ratio of nuclides A and B in the troposphere at time t, $A_{T,0}$ and $A_{S,0}$ are the amounts of A in the troposphere and in the stratosphere at $t=0$, k_T and k_S are the reciprocals of the mean storage time of the fission products in the troposphere and in the stratosphere, respectively, $(B/A)_{T,0}$ is the B/A ratio in the troposphere at $t=0$, and $(B/A)_{S,0}$ is the B/A ratio in the stratosphere at $t=0$.

Suppose that $A_{T,0-}$ and $A_{S,0-}$ of the nuclide A were present in the tropo-