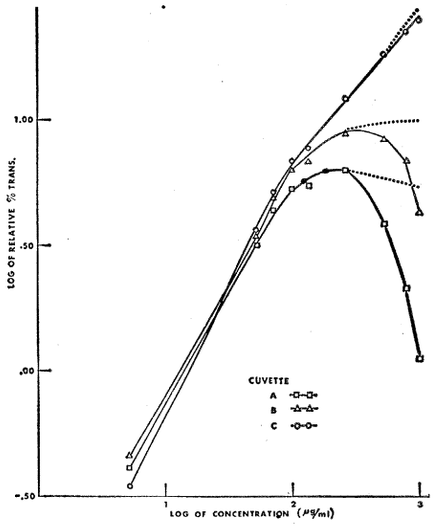

Fig. 1. The log of relative percent transmission (F') versus the log of concentration for various sizes of cuvettes: "A," 46 mm by 10.5 mm square; "B," 40 mm by 7 mm round; "C," 20 mm by 3 mm square; at peak excitation of 285 mμ and peak emission of 350mμ in 0.1N sulfuric acid for morphine.

ducible at a particular wavelength over some range of concentration for below-concentration quenching dilutions.

For the same compound at a particular concentration, F' is proportional to the "effective" optical light path, and as b increases the value of $(1 - 10^{-b})$ approaches unity (Eq. 2). The "effective" optical light path can, in theory, be lengthened by increasing the cuvette width.

$$F' = K\ (1 - 10^{-b}) \qquad (2)$$

where K is the factor for the multiple values, ε, ϕ, I_0, and c.

With the use of 0.1N sulfuric acid solutions of morphine, the 285 mμ peak was used as the major activation to excite maximum emission at 350 mμ (*4*). A second excitation peak observed at 245 mμ may be the actual major one, but the rapid falloff of I_0 below 280 mμ for the xenon lamp would mask this. The plot of the log of F' versus the log of c was previously shown (*4*) to be linear from 0.1 to 100 μg/ml with solutions in the "A" cuvette (described below). At higher concentrations there was a marked decrease in fluorescence which may be interpreted as being concentration quenching of the ionic dimer type theorized by Forster (*5*).

For true quenching it would be expected that a plot of concentration versus fluorescence would be linear for dilute solutions, reaching a maximum with increasing concentrations, and finally decreasing with further change in concentration. The measurement of fluorescence emission at angles less than 90° to the exciting source results in a similar plot, but the range of linearity is extended by several magnitudes (*6*). Since most available spectrofluorometers and fluorometers are used to measure emission at either 90° to the exciting radiation or directly through the solutions, a special instrument or modification (*6*) of existing instruments would have to be used to show that reabsorption at higher concentrations is responsible for "apparent" concentration quenching. In place of modifications, the light path was increased simply by using wider cuvettes.

Various cuvette sizes (*7*) are available with adaptors for the Aminco-Bowman instrument. It was expected that at concentration quenching ranges the fluorescence would decrease in the order of the decrease of cuvette width: "A," 46 mm by 10.5 mm square; "B," 40 mm by 7 mm round; "C," 20 mm by 3 mm square. This would obey Eq. 2. The lens effect for the round cuvette is probably slight for the narrow slits used in these studies. The results shown graphically in Fig. 1 indicate that with the smallest cuvette "C," the F' is greater at high concentrations. Therefore, an absorption effect, and not concentration quenching, accounts for the decreased F' for longer b. Linearity is noted for the "C" cuvette at 100 μg/ml until close to 1000 μg/ml. This is an extension of the utilizable fluorescence range. At lower concentrations, the emission from the "C" cuvette begins to correspond to Eq. 2, and at 5 μg/ml, the emission yield is below that of either of the two others used, thus showing the validity of Eq. 2 for this range.

Excitation peak shifts to higher wavelengths are observed as concentration increases. These shifts of intensity are shown as dotted lines in Fig. 1 and are caused by the same inner filter effect that yields "apparent" concentration quenching. A compound with an absorbance of 0.02 would introduce an inner filter error of about 4 percent (*1*). As the absorption increases, this error increases, owing to the failure of excitation of a large portion of the molecules present. This same phenomenon is dramatically noted in the visual observation of fluorescein solutions at high concentration (10^{-4} to $10^{-5}M$). In obtaining absolute quantum efficiencies (*8*), this effect is avoided by surface emission detection.

The possibility for the oxidation of morphine to pseudo-morphine at room temperature (25°C) in acid is eliminated in these measurements by preparing solutions directly before measurement (*9*; *10*).

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References and Notes

1. C. A. Parker and W. T. Rees, *Analyst* **85**, 587 (1960).
2. D. M. Hercules, *Science* **125**, 1242 (1957).
3. C. E. White, M. Ho, E. Q. Weiner, *Anal. Chem.* **32**, 438 (1960).
4. R. Brandt, S. Ehrlich-Rogozinsky, N. D. Cheronis, *Microchem. J.* **5**, 215 (1961).
5. T. Forster, *Fluoreszens Organischer Verbindungen* (Vandenhoeck and Ruprecht, Gottingen, 1951), p. 252.
6. T. J. Porro, W. Slavin, B. Gillette, *A Versatile Micro Spectrofluorimeter Accessory* (Perkin-Elmer Corp., Norwalk, Conn.).
7. Cuvettes used: "A," Cat. No. 4–8110; "B," Cat. No. 4–8112; "C," Cat. No. 4–8114; American Instrument Co., Silver Spring, Md.
8. W. H. Melhuish, *J. Phys. Chem.* 65, 229 (1961).
9. S. Y. Yeh and J. L. Lach, *J. Pharm. Sci.* **50**, 35 (1961); *Am. J. Hosp. Pharm.* **17**, 101 (1960).
10. Supported by research grant DA-CML-18-064-62-G25 from the U.S. Army Chemical Corps, Biological Labs, Fort Detrick, Md.
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3 January 1963

Strontium-90 in Hair

Abstract. *The hair of rats injected with strontium-90 retains a significant amount of the radionuclide. Although the strontium-90 content of hair is variable in these rats and appears to be subject to a variety of influences, determination of the radionuclide content of hair may offer a nondestructive method of estimating strontium-90 in bone.*

Since hair contains strontium as well as a variety of other elements (*1*), we decided to determine whether strontium-90 accumulates in this tissue to an appreciable degree. Accordingly, we assayed the hair of rats with varying body burdens of Sr^{90} and found that their hair contained significant amounts of this radionuclide.

Hair was clipped from groups of 4 to 12 adult rats whose body burden of Sr^{90} had been established by injection on the 300th day of life, or *in utero*. In the latter cases the mothers were injected on the 17th or 20th day of pregnancy.

A suitable number of uninjected rats from the same colony were used to provide control hair. The hair was usually taken from the back, but in some cases the rat was clipped almost completely. Approximately the same amount of hair was taken from each rat.

The clippings were combined by groups, weighed dry, washed with water containing a detergent, rinsed well with tap water, and finally rinsed with 1*N* HCl. This treatment removed surface dust, oils, and other materials from the hair. Subsequent assay of the wash liquors showed that surface contamination with Sr^{90} was negligible.

The washed samples were dry ashed in a muffle furnace at 600°C. The ash was taken up in 0.5*N* HCl, and the solution was brought to a standard volume. One-milliliter aliquots were measured in a deep-well counter calibrated against a U.S. Bureau of Standards Sr^{90}-Y^{90} solution, and the Sr^{90} content of the hair was calculated.

The Sr^{90} content of the pooled samples of hair from control and experimental rats is compared with the body burdens in Table 1. The control hair had no activity, corresponding to the body burden, but the hair from the injected rats showed variable levels of Sr^{90} activity. The highest values were found in the hair from the groups in which the body burden had been established in adult life 0.6 to 2.0 months prior to the determinations. A level of 65,200 pc per gram was found in the hair of rats with an average body burden of 57.8 μc 0.6 month after injection. In contrast, the hair of rats with body burdens established *in utero* 4.3 to 5.7 months prior to the determinations showed levels of activity varying from 320 to 960 pc per gram.

The lower level of activity in the hair of the rats with body burdens established *in utero* may be due largely to loss of hair during growth from birth to adulthood, during which four to five hair growth cycles may have occurred (*2*). Sternberg (*3*) observed in his study of the distribution of Sr^{90} in guinea pigs that an average of 0.06 percent of the injected nuclide appeared in the hair within 24 hours after administration whether the dose was given orally or subcutaneously. Accordingly, the transfer of the nuclide to the hair must be rapid. It seems probable that autoradiographs of rat hairs taken at intervals after injection of Sr^{90} might show the rate at which the nuclide is incorporated into the hair.

Our studies do not show the form in which the strontium is present in the hair, and this might be difficult to determine because of the extremely low concentration. It is known from the work of LeBlond (*4*) that iodine in hair is present as inorganic iodide, and from the work of Brown-Grant and Pethes (*5*) that radioiodine accumulates in the hair to the extent of 0.05 percent of the injected dose per gram of tissue 250 hours after intramuscular administration of sodium iodide-131. Similarly, Ballou and Thompson (*6*), in their comprehensive investigation of the metabolism of zinc-65 in rats, have observed that animals chronically dosed for 407 days with zinc-65 show hair values of 27 percent of the daily dose per gram of tissue against femur values of 12 percent. Indeed, the thyroid-seeking iodine-131 and the bone-seeking zinc-65 show high values in hair compared with other tissues.

The biological mechanisms by which various radionuclides are incorporated in hair evidently differ. Ryder (*7*) showed that rats and mice injected with copper-64 of half life 12.8 hours, and killed 1 to 13 hours later, did not localize the nuclide in the hair follicle, whereas they did localize radioiodine and radiozinc there. Since the copper of ascorbic acid oxidase cannot be exchanged for cupric ion in solution (*8*), a copper bonding in hair equivalent to covalent bonding may explain Ryder's results (*7*). As mentioned above, hair seems to contain iodine as inorganic iodide, but zinc, strontium, and other polyvalent metals may be organically bound. To define these relationships to hair elements, comparative studies with rats are in progress, on the incorporation of several isotopes in plucked as well as clipped hair, and in freshly grown hair on skin plucked prior to the injection of the radionuclides. Autoradiographs may be helpful in this study of nuclide distribution in the follicle.

Table 1. Strontium-90 content of hair.

Av. body burden per rat (μc)	Rats in group (No.)	Skeletal exposure (mo.)	Hair (g)	Sr^{90} per gram of hair (pc)
		Control		
0	8		37.3	0
0	10		38.1	0
		Established during adult life		
57.8	11	0.6	18.0	65,200
36.0*	4	2.0	9.0	8,200
		Established in utero		
2.0†	4	4.3	17.3	320
0.5‡	8	5.7	28.2	960
0.1†	12	5.7	41.0	360

* The average body burden decreased from 54 μc to 17 μc during the 2-month period. † Mothers of this group were injected on the 17th day of gestation. ‡ Mothers of this group were injected on the 20th day of gestation.

Although these preliminary studies suggest that analysis of hair may furnish a nondestructive method of measuring bone-seeking radioelements, much additional work is needed to define the limitations of the method, especially as it applies to man. It seems doubtful that hair, or hair ash, from herbivores, such as cattle or sheep, or from man, contains enough Sr^{90} from fallout to be detected directly by the methods used in this work, where the counting efficiency was about 10 percent. But procedures for concentrating radionuclides are so well developed that hair could be employed for monitoring Sr^{90} fallout, if desired. Since most reports (*9*) on Sr^{90} fallout are expressed as picocuries of Sr^{90} per gram of calcium, additional measurements on the calcium content of hair are required. The data (*1*, *10*) on the calcium content of human hair indicate a range of 188 to 4900 parts per million, but there are few determinations on the calcium content of the hair of laboratory and domestic animals. Similarly, the variations in the stable strontium content of hair are not well defined for either man or animal. Until values are available on the stable strontium and calcium content of hair, the Sr^{90} content of hair and the Sr^{90}/Ca ratios cannot be studied completely (*11*).

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References and Notes

1. P. Flesch, in *Physiology and Biochemistry of the Skin*, S. Rothman, Ed. (Univ. of Chicago Press, Chicago, 1954), p. 642.
2. M. P. Mohn, in *The Biology of Hair Growth*, W. Montagna and R. A. Ellis, Eds. (Academic Press, New York, 1958), pp. 336–398.
3. J. Sternberg, "Tissular distribution and placental transfer of strontium-90 in pregnant guinea pig," in *Radioaktive Isotope in Klinik und Forschung* (Urban and Schwarzenberg, Munich-Berlin, 1960), vol. 4, pp. 73–92.
4. C. P. LeBlond, *Endocrinology* **54**, 104 (1954).
5. K. Brown-Grant and G. Pethes, *J. Physiol.* **148**, 683 (1959); **152**, 474 (1960).
6. J. E. Ballou and R. C. Thompson, *Health Phys.* **6**, 6 (1961).
7. M. L. Ryder, in *The Biology of Hair Growth*, W. Montagna and R. A. Ellis, Eds. (Academic Press, New York, 1958), pp. 305–334.
8. M. Joselow and C. R. Dawson, *J. Biol. Chem.* **191**, 11 (1951).
9. For example, J. L. Kulp and A. R. Schubert, *Science* **136**, 619 (1962).
10. W. S. Spector, Ed., *Handbook of Biological Data* (Saunders, Philadelphia and London, 1956), p. 77.
11. Aided in part by research grant R-42, Division of Radiological Health, U.S. Public Health Service.

9 January 1963