tribution to the calcium intake is trivial and because the Sr^{90}/Ca ratio is not expected to exceed that in milk by more than a factor of 2.

The chemical and radiometric procedures have been described elsewhere (*3*). The over-all yield of strontium was monitored with a Sr^{85} tracer. A representative set of six frozen vegetables was prepared according to the directions on the package, and the liquid phase was analyzed separately. No appreciable Sr^{90} is removed in the preparation of the vegetables for human consumption.

The data on U.S. milk (Table 3) include those of the Health and Safety Laboratory of the AEC New York Operations Office (*4*), extrapolated to late 1957 where necessary. The variations in Sr^{90} concentration from one farm to the next are probably related to the available calcium content of the pasture and to the average root depth of its grass. Duplicate milk samples from two nearby farms in Virginia gave 1.9 and 1.9, and 8.1 and 7.1 μμc of Sr^{90} per gram of calcium (hereafter referred to as strontium units, SU), respectively. Variations up to a factor of 2 occur from a single distribution source (Bergen County, N.J.) over a period of a month, reflecting changes in relative quantities of milk from contributing farms in successive batches. Despite these short-time variations, the average monthly value for different parts of the country is quite uniform, giving an average concentration for the country of about 6 SU. In comparison, the average level of Sr^{90} in British milk would be 7 to 8 SU in late 1957, on the basis of an extrapolation of the 1956 data (*5*).

The vegetables and cereals (Tables 1 and 2) are representative of large-acreage production. Variations from one sample to another grown in the same general area probably reflect different soil conditions. No appreciable increase in Sr^{90} from mid-1956 to early 1957 is observable from the data, as is not wholly unexpected, since an increase in Sr^{90} in the total fallout was only about 20 percent during this period.

Geographical differences in the Sr^{90} concentration appear but do not exceed two times the mean. In the diet, however, these differences are averaged out because of the nature of commercial food distribution. Some differences appear among plant types—for example, asparagus is relatively low, but among the major calcium contributors (peas, beans, and cereals), the Sr^{90} level is rather uniform.

The U.S. population obtains 85 percent of its calcium from milk, 4 percent from cereals, and 5 percent from vegetables (*6, 7*). If the average concentration of Sr^{90} in these foods in the United States in late 1957 is assumed to be 6, 15, and 10 SU, respectively, the average diet contains about 6.5 SU. In an extreme case, a vegetarian might have double this value.

Monthly integrated tap-water samples in the New York City area now carry about 0.1 μμc of Sr^{90} per liter. If an average consumption of 1 liter of water and 1 g of calcium from food each day is assumed, the contribution of Sr^{90} from drinking water appears to be negligible. If rain water were consumed, this source would still only account for about 20 percent of the daily Sr^{90} intake.

It is concluded that the Sr^{90} content in the diet of an average U.S. citizen in 1957 was about 6.5 SU, corresponding to an equilibrium base level of 1.6 SU, since the discrimination factor between diet and base appears to be about 4 (*1, 5*). If the diet concentration remains constant for a decade, the equilibrium bone level of 1.6 SU would be approached by young children. New-born children would have about half of this value on account of fetal discrimination, and adults would reach only 20 to 30 percent of the equilibrium level, because of the slow rate of exchange of the calcium in bone.

Table 3. Strontium-90 in milk from various locations, 1956–57. The numbers in parentheses in column 3 give the number of samples.

Location	Date	SU	
New Jersey (Bergen County)	9–10/57	Range	3.0–7.7
		Av.	5.5 (14)
New Jersey (other)	10/57	Av.	5.5 (4)
New York City (retail)*			5.5
New York State (Perry)*			4.5
Mohawk Valley	9–10/57	Av.	6.6 (4)
North Carolina	8/57	Av.	5.3 (4)
North Dakota (powdered)*			10.0
Mississippi (State College, powdered)*	?/56		6.5
Missouri (St. Louis, powdered)*			6.5
Oregon (Portland, powdered)*			7.0
Virginia (Rockingham County)	10/57	Av.	3.8 (4)
Wisconsin (Columbus, powdered)*			5.5
Av. for all 1957 milk			6.1

* Estimated from an analysis reported by Health and Safety Laboratory, New York Operations Office of the AEC (*4*), extrapolated to late 1957.

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17 February 1958

Effect of Zinc on the Determination of Cyanide with Phenolphthalin

Determination of cyanide by the oxidation of phenolphthalin in alkaline solution to phenolphthalein in the presence of a trace of cupric salt and cyanide (*1*) is a method that has been used to determine trace amounts of cyanide in a wide variety of biological and industrial media. The determination is not specific for cyanide but is subject to interference by various oxidizing substances (*2*). Recent work on plating-room wastes in which this method was used has raised the question of the effect of zinc in such solutions. Zinc can form stable cyanide complexes which might cause negative errors.

To ascertain whether or not such errors exist (*3*), a series of known cyanide solutions was made up, and known amounts of zinc were added. This method of determining cyanide consists of adding 2 ml of the unknown to 10 ml of 0.05-percent KOH, followed by 10 ml of indicator solution. The indicator solution consists of 99.5 ml of 1-percent $CuSO_4 \cdot 5H_2O$ plus 0.5 ml of 1-percent phenolphthalin dissolved in 0.67-percent NaOH. This indicator solution is not stable and should be made up fresh every 2 hours. Absorbance is read at 553 mμ, the absorbance peak for phenolphthalein (*4*), 3 minutes after the indicator solution has been added. The calibration curve for cyanide alone is a straight line passing through zero.

One might suppose that, if zinc does complex cyanide with a stability sufficient to interfere with this method of determining cyanide, absorbance would decrease as zinc content increases, falling to zero with the addition of an amount of zinc proportional in some manner to the amount of cyanide present. However, Fig. 1 shows that, as the zinc content is increased, the absorbance increases to a flat maximum, then rapidly falls to zero with addition of a specific amount of zinc which is independent of the amount of cyanide present. The increases in absorbance are neither constant nor proportional to the absorbance in the absence of zinc.

Since the cyanide solutions are basic and the zinc solutions are acidic, the effect of *p*H was briefly investigated. Solutions of NaOH and HCl were made up which matched the zinc and cyanide stock solutions with respect to *p*H. A colored cyanide solution is decolorized by HCl and recolored by NaOH. After the initial addition of HCl, a single drop of base or acid is sufficient to change the color, as one would expect at the end point in an acid-base titration in which phenolphthalein is used as the indicator.

On the other hand, a cyanide solution which has been decolorized with zinc can be recolored by adding cyanide, but more than three times the amount of cyanide originally present is required, and the second color is weaker than the first. This second color can then be discharged with about one-tenth the amount of zinc first required. Subsequent recolorization and decolorization requires an excess of cyanide and only a small amount of zinc.

It is thus apparent that the phenolphthalin method for determining cyanide is subject to interference by zinc. Often when an ion interferes with an analytical method, the method can be adapted as a means of analyzing for that ion. The shape of the curves in Fig. 1 indicates that this cannot be done in the present case. However, by standardizing cyanide in the presence of, say, 6 parts per million of zinc, one would be operating in the flat portions of the curves, and then one could determine cyanide in the presence of zinc without error, as long as the zinc concentration did not vary beyond the limits of 3 to 10 parts per million.

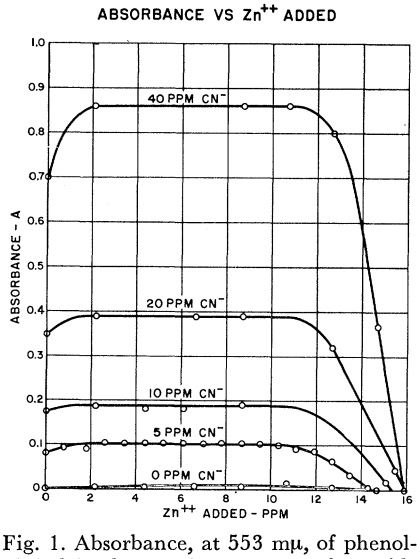

Fig. 1. Absorbance, at 553 mμ, of phenolphthalein due to the presence of cyanide versus amount of zinc added.

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Reactivation of Cytochrome Oxidase by Lipide

There are reports in the literature which indicate that lipides or lipide-soluble substances may be important for the normal activity of respiratory enzymes. For example, Nason and Lehman (*1*) have investigated the restoration by tocopherol of DPNH- and succinate-cytochrome *c* reductase activity of rat skeletal muscle following isooctane extraction and aging to reduce the activities. Martius and Nitz-Litzow (*2*) have proposed that vitamin K_1 is a necessary component of the DPNH-cytochrome *c* reductase system. They have based their hypothesis on (i) the inhibitory effect on oxidative phosphorylation of dicoumarols and related compounds, (ii) the reduced phosphorylation in mitochondria from the livers of vitamin K-deficient chicks (which is restored by vitamin K_1), and (iii) the identification of a DPN-dependent vitamin K_1 reductase in pig liver mitochondria.

More recently Crane, Hatefi, Lester, and Widmer (*3*) have reported the isolation from beef heart mitochondria of a quinone capable of undergoing reversible oxidation and reduction. This compound, with an absorption maximum at 275 mμ (designated as Q-275), will reactivate the succinoxidase system of heptane-extracted mitochondria, and in the reduced state can be oxidized by ferricytochrome *c* in the presence of the electron-transport particle. Previous reports that implicate a lipide in the cytochrome *c* oxidase portion of the chain include those that demonstrate an inactivating effect of lecithinases (*4*). More recently, Witter, Morrison, and Shepardson (*5*) have found that lysolecithin uncouples phosphorylation from oxidation of ascorbate-cytochrome *c* by the cytochrome oxidase contained in rat liver mitochondria and thus enhances oxygen uptake by about 100 percent.

In the course of attempting to purify the cytochrome oxidase of beef heart mitochondrial fragments (*6*) by extracting with surface active agents, we found that, after treatment of the particles on a cellulose column with deoxycholate (1.9 percent) and with cholate (4.0 percent), the cytochrome oxidase that was finally solubilized with 3 percent deoxycholate (S3) was relatively inactive. The addition of the 4 percent cholate extract (S2), however, reactivated some of the enzyme (Table 1, experiment 1). In another experiment the 1.6-percent deoxycholate extract (S1) proved to be an even better activator (Table 1, experiment 2) of a slightly active 2.5-percent deoxycholate extract (S3).

The activating substance, free of surface-active agents, proved to be heat stable and extractable by butanol but not by ethyl ether or petroleum ether. A number of lipides and lipide-soluble substances were tested for their capacity to activate the enzyme. The following compounds were ineffective: oleic acid, vitamin K_1, cholesterol, DL-α-tocopherol phosphate, choline, and phosphoryl choline. In Table 1, experiment 3, are presented those compounds which proved to be effective activators. There are several possibilities to be considered for the activating effect by these phospholipides:

Table 1. Reactivation of cytochrome oxidase. Cytochrome oxidase activity (*9*) is expressed as the first-order velocity constant.

Fraction	Activity ($\times 10^{-3}$ sec^{-1})
Experiment 1	
S3	0.47
S2	0
S3 + S2	1.34
Experiment 2	
S3	0.69
S1	0.75
S3 + S1	8.54
Experiment 3	
S3	0.93
S3 + animal lecithin (pract.)	4.85
S3 + animal lecithin (purified by chromatography)	1.60
S3 + vegetable lecithin	2.45
S3 + cephalin (impure)	2.80
S3 + dimyristoyl lecithin	2.88