Radiation Comparison of Cobalt Isotopes

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THE VALUE of radioactive vitamin B_{12}[1-5] has been established as a diagnostic agent in pernicious anemia and related diseases, as a tracer for this vitamin in animal and *in vitro* studies, and as an analytic agent for certain types of isotope dilution procedures and biologic potency determinations. The radioactive constituent is a cobalt isotope, the properties of which determine the sensitivity of detection of the vitamin and the radiation dose which a test subject receives.

HALF-LIFE

Factors which influence the choice of radioactive isotope are (1) half-life (T), (2) availability, (3) specific activity (radioactivity per unit weight), and (4) the nature and energies of radiations emitted. Of about twelve radioactive cobalt isotopes reported,[6] all but four are eliminated because their rates of radioactive decay are inconveniently short, i.e., reported half-lives range between 0.2 second and 18 hours. The four remaining isotopes, those with atomic mass numbers 56, 57, 58 and 60 and respective half-lives of 77 days, 270 days, 72 days and 5.27 years, have all been successfully employed for the labeling of vitamin B_{12}. Their decay rates are sufficiently slow to yield useful products, despite the time required for microbiologic synthesis, isolation and purification of the labeled vitamin.

AVAILABILITY

$Cobalt^{60}$ has been used most frequently because it is in constant supply and readily obtainable from the U. S. Atomic Energy Commission[7] and comparable regulatory agencies in other countries producing and distributing radioisotopes. It is produced in a nuclear fission reactor as a result of neutron (n) capture by stable cobalt (mass number 59), with simultaneous emission of gamma radiation. This (n, γ) reaction is the most common type of neutron reaction and may be represented as $Co^{59}(n, \gamma) Co^{60}$.

$Cobalt^{58}$ is also supplied by the U. S. Atomic Energy Commission.[7] It is also prepared in a fission reactor, but from separated isotope $nickel^{58}$ by neutron capture with emission of a proton (p), i.e., by the reaction $Ni^{58}(n, p) Co^{58}$. Unfortunately, a trace of $nickel^{60}$ present in the nickel target gives rise to contamination by $cobalt^{60}$ which, although unimportant initially, becomes more evident as the $cobalt^{58}$ decays. A trace of iron isotope Fe^{59} is also present in $cobalt^{58}$.

In contrast to the fission reactor source of the preceding isotopes, $cobalt^{56}$ and $cobalt^{57}$ are prepared in an ion accelerator such as the cyclotron. $Cobalt^{56}$ is produced by bombarding iron targets with deuterons (d) or protons which lead to mixtures of $cobalt^{56}$ and $cobalt^{57}$ by nuclear reactions such as $Fe^{56}(d, 2n) Co^{56}$ and $Fe^{56}(d, n) Co^{57}$. Similarly, $cobalt^{57}$ may be formed by interaction of a proton beam with highly purified nickel (free of cobalt and iron contamination) in which the principal reaction induced is $Ni^{58}(p, pn) Ni^{57}$ followed by rapid decay of $nickel^{57}$ to $cobalt^{57}$. Although $cobalt^{56}$ has been available in limited quantities for some time, supplies of $cobalt^{57}$ have only recently become assured.[8]

SPECIFIC ACTIVITY

Detection sensitivity is determined primarily by the specific activity of the radioactive substance. The specific activity of radioactive vitamin B_{12} is determined by the specific activity of the cobalt present initially in the

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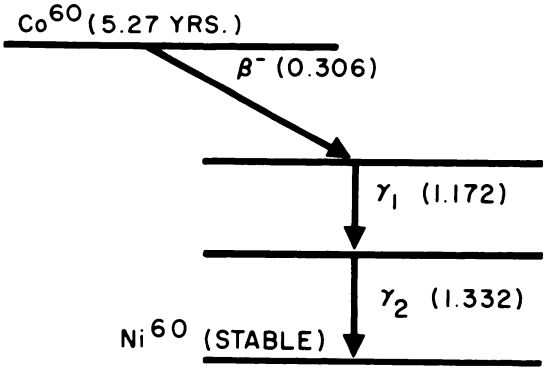

FIG. 1. Decay scheme for cobalt[60].

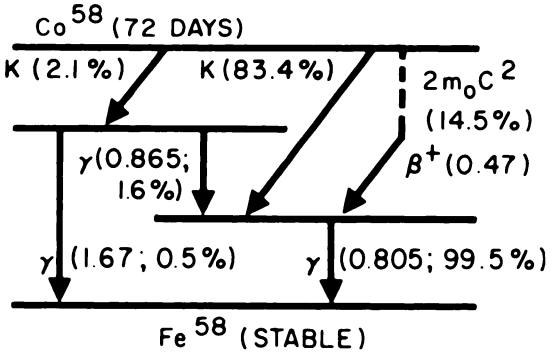

FIG. 2. Decay scheme for cobalt[58].

medium in which microbiologic synthesis of the vitamin occurs. Although metallic cobalt sources are offered by the U. S. Atomic Energy Commission[7] with activities up to 50 mc./mg. Co, the most active cobalt[60] received by Merck & Co., Inc., in the form of soluble cobalt salt, has had at best a specific activity of ≃20 to 25 mc./mg. Accordingly the highest vitamin B_{12}-Co^{60} activity attainable is ≃1 mc./mg. since the cobalt content of the vitamin is 4.34 per cent. This is the specific activity of the radioactive vitamin marketed and can be exceeded only if cobalt[60] of higher activity is provided. In such a preparation, approximately 1 cobalt atom per 50 is radioactive (i.e., Co^{60}).

No such limitation attaches to labeling of vitamin B_{12} with Co^{58}. This isotope is supplied in "carrier-free," essentially isotopically pure form, and must be diluted only to the extent that exogenous cobalt is required by a particular strain of microorganism to produce a suitable yield of vitamin. It has been shown,[9] for example, that a strain of Streptomyces griseus produces a maximum yield in a nutrient medium containing 1 to 2 p.p.m. cobalt, although significant yields of vitamin have been produced at cobalt concentrations of 0.05 to 0.1 p.p.m. Since cobalt[58] is essentially weightless, even in quantities of 20 mc. (equivalent to 0.64 μg. Co), stable cobalt (cobalt[59]) must be added to a medium to insure a reasonable yield. This balancing of size batch and yield with resultant specific activity led Merck & Co., Inc., to offer a product of 5 mc./mg. vitamin B_{12}.

Similar considerations would apply to cobalt[56] and cobalt[57]. Vitamin labeled with the former isotope is available from Glaxo Laboratories in London. Specific activities[5,10] of 12 mc./mg. of vitamin B_{12}-Co^{56} and 13.5 mc./mg. of vitamin B_{12}-Co^{57} have been reported. In 1950 we produced a $Co^{56,57}$-labeled preparation for use in human subjects by Dr. Marshall Brucer, Oak Ridge Institute of Nuclear Studies. Cobalt[57] of high specific activity and isotopic purity has not been available in quantity until recently.[8] It would be desirable to have a source of vitamin B_{12}-Co^{57}, with an activity of ≃5 mc./mg. for use by investigators. With cobalt[57] available, this becomes a distinct possibility. The superiority of cobalt[57] over cobalt[58] in terms of useful life-time is evident.

In cobalt-labeled vitamin with a specific activity of 5 mc./mg., there is present approximately 1 cobalt[56] or 1 cobalt[58] atom per 300 total cobalt atoms, while in the form labeled with cobalt[57], the ratio is 1 per 85.

DECAY SCHEMES

The types, energies and sequence of emission of radiation by a radioactive nucleus are depicted diagrammatically in what is called a decay scheme. Figures 1 through 4 are the accepted diagrams for the four isotopes in question. Exact values of branching or energies may be subject to some revision, but the following considerations will not be effected significantly. Cobalt[60] is seen (Fig. 1) to emit in cascade one beta (β^-) ray (0.306 Mev) and two gamma (γ) rays (1.172 and 1.332 Mev) per disintegration.[11] Cobalt[58] is seen (Fig. 2) to undergo branching decay,[12,13] i.e., 14.5 per cent of disintegrations proceed by positron (β^+)

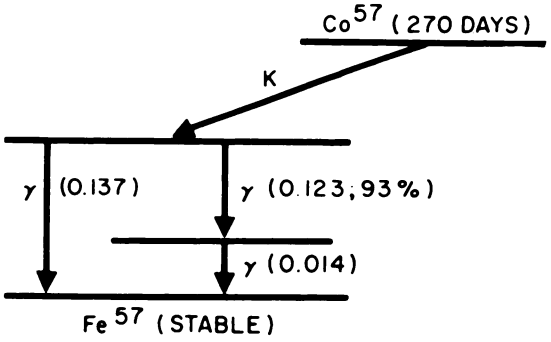

FIG. 3. Decay scheme for cobalt[57].

emission (0.47 Mev) while 85.5 per cent are accompanied by electron capture (K). Both modes of decay are followed by emission of gamma rays (0.805 Mev, 99.5 per cent; 0.865 Mev, 1.6 per cent; 1.67 Mev, 0.5 per cent). In addition, annihilation of each positron produces two 0.51 Mev gamma rays. Cobalt[57] (Fig. 3) decays[14] entirely by electron capture, followed by branched emission of gamma rays of 0.137 Mev ($\simeq$7 per cent of disintegrations) and by a sequence of 0.123 and 0.014 Mev rays (93 per cent). Cobalt[56] has the most complicated decay pattern[13,15,16] (Fig. 4), emitting eleven gamma rays plus annihilation radiation following 19.2 per cent decay by positron (1.47 Mev) emission and the remainder by a complex combination of electron capture processes. The eleven different gamma rays emitted, exclusive of annihilation (two 0.51 Mev rays per β^+ emission) are listed in Table I, which also shows the number (in per cent columns) of the several gamma rays produced per 100 disintegrations.

TABLE I
Emission of Gamma Rays by Co[56]

Mev	Per Cent	Mev	Per Cent
3.47	1.1	1.35	5.7
3.25	11.5	1.22	69.6
2.98	1.9	1.025	15.6
2.56	15.4	0.975	1.8
2.02	11.4	0.845	98.9
1.76	16.7	—	—

DETECTION SENSITIVITY

With the exception of cobalt[57], each of these isotopes can be detected by conventional Geiger tubes sensitive to particulate radiation. Of these isotopes, cobalt[60] is most readily detected since each disintegration is accompanied by ejection of a beta particle. Despite the emission by cobalt[58] and cobalt[56] of positrons of greater energy than that of the cobalt[60] beta particle, positron emission accounts for only a fraction (14.5 and 19.2 per cent) of the branching; and, for equal radioactivities, beta counting is a more sensitive procedure for cobalt[60] than for the other two nuclides. This would apply to liquid scintillation methods as well as to gas discharge tubes.

This superiority is not maintained in the solid scintillation counting device commonly employed in clinical laboratories. Gamma radiation (as in cobalt[60]) of high energy penetrates matter more readily but is less readily absorbed by a phosphor such as NaI(Tl) than is less energetic gamma radiation as is emitted by cobalt[58] or cobalt[57]. Thus the greater attenuation of gamma radiation from the latter isotopes, as compared to that from cobalt[60] and cobalt[56], during the passage of radiation through a given medium (i.e., tissue or sample) is compensated in part by greater absorption in the phosphor crystal. Furthermore, the thallium-activated NaI scintillation crystal has a maximum scintillation efficiency[17] at $\simeq$0.1–0.15 Mev which is extremely favorable to cobalt[57]. One would expect that, in the absence of a great depth of absorbing medium and where thin NaI(Tl) crystals are employed, gamma scintillation detection efficiency would be greatest for cobalt[57] and least for cobalt[60], with cobalt[58] probably intermediate. An accurate intercomparison has not yet been reported and results would depend on the conditions of measurement.

MAXIMUM PERMISSIBLE CONCENTRATIONS

Safety requirements impose limits on the amounts of a radioisotope which may be administered to a human subject. Maximum permissible body concentrations (MPC) are computed on the basis of the known distribution of isotope in critical organs and an accepted permissible whole body radiation dose rate such that blood-forming organs receive no

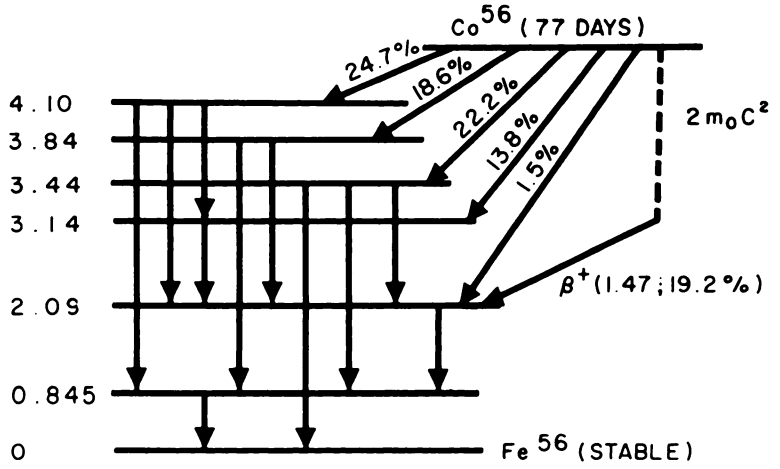

FIG. 4. Decay scheme for cobalt[56].

more than 300 milliroentgens (mr.) per week. This official limit is set by the U. S. Atomic Energy Commission for occupational exposure,[18] and is based upon figures published in its Handbook 52 by the National Bureau of Standards.[19] Although revisions of these limits have been recommended by the National Committee on Radiation Protection and Measurements, these have not been officially adopted as of this symposium date; and all maximum permissible tissue concentrations will be computed upon the current basis of 300 mr./week.

According to Handbook 52, the maximum permissible total number of microcuries of a given isotope which will result in a dose rate* of 300 rep/week is

$$q = \frac{2.6 \times 10^{-3} \, m \, W}{\Sigma \, (b \, E) \, f_2}$$

where m is the mass (1,700 gm. for liver) of the critical organ, Σ (b E) is the effective (absorbed) energy of radiation (of E in Mev units) per disintegration, and f_2 is the fraction of total body isotope which is present in the target organ. Absorption coefficients of beta rays, positrons and gamma rays are included in computations of b as described in the handbook. The effective liver diameter is taken as 10 cm. If W is 0.3 rep/week adopted as maximum permissible radiation dose rate, q becomes equal to the MPC in microcuries. Computed values of Σ(b E) and q for the four cobalt isotopes in question are listed in Table II on the assumption that we are dealing with cobalt concentrated in the liver as the target organ with an f_2 of 0.68. These MPC's (q) represent the number of microcuries of cobalt isotope which will deliver radiation to the target organ at a dose rate of 300 rep/week. Obviously, the isotope tolerable in largest amount (of radioactivity) is cobalt[57], (MPC of 40 μc. compared to the small MPC value of ≃2 μc. for cobalt[56] and ≃3 μc. for cobalt[60]).

This in itself is not, however, the sole criterion of usefulness, since these are instantaneous values and do not take into account the decay rates of these isotopes which determine the total absorbed radiation energy, and the frequency with which repeated doses may be administered. Column 4 of Table II contains values of total rep delivered to the liver during complete decay of the MPC's (in the absence of biological elimination or turnover). These integrated doses are equal to (0.3 × T)/0.693, where T is the effective half-life, here taken as the radioactivity half-life in weeks. The actual total exposure should take into account the biologic half-life (Tb) of the element. This is given as 8.4 days for cobalt in the liver. Accordingly, the total doses delivered to the

* The roentgen (r) applies strictly only to x- and gamma rays. For isotopes which emit particulate radiation, the term "roentgen equivalent physical" (rep) applies. In this discussion, no distinction is made between r and rep since all radiations emitted by the cobalt isotopes are considered to have the same relative biologic effectiveness.

TABLE II
MPC and Cumulative Exposures Due to Radioactive Cobalt Isotopes

Isotope	Σ(b E)	MPC (μc.)	Total rep to Liver Containing MPC				
			Tb Infinite	Tb = 600 Days	Tb = 400 Days	Tb = 180 Days	Tb = 8.4 Days
Co^{56}	0.963	2.0	4.8	4.2	4.0	3.3	0.47
Co^{57}	0.0488	40.0	16.7	11.5	10.0	6.7	0.50
Co^{58}	0.293	6.7	4.5	4.0	3.8	3.2	0.46
Co^{60}	0.736	2.7	117.0	28.0	20.0	10.0	0.52

liver during the effective half-life* of the isotope, if present as elemental cobalt, would be those compiled in the last column of Table II. Between this and the column (fourth) representing absence of elimination are listed cumulative rep values for Tb = 180, 400 and 600 days, which pertain more properly to cobalt administered in the form of vitamin B_{12}.

It is clear that the most favorable (largest) MPC is 40 μc. of Co^{57} and the smallest is 2 to 3 μc. for Co^{56} and Co^{60}. This is primarily a question of type of radiation, or differences in Σ bE values and, in the case of Co^{57}, reflects in part the absence of particulate radiation. $Cobalt^{57}$ thus becomes the preferred cobalt isotope from the point of view of radiation safety,† even when a q comparison is made of permissible microcurie burdens for equal cumulative doses. Because of the difference in decay rates, the cumulative dose delivered to tissue is potentially greatest for $cobalt^{60}$, which is thus the least desirable of the isotopes for use in human subjects. For cobalt salts, however, with a biologic half-life of 8.4 days, rapid turnover equalizes the radiation dosage delivered by MPC's of the several isotopes, although the values of MPC remain unchanged.

CASE OF RADIOACTIVE VITAMIN B_{12}

For longer terms of residence in the body as is known to be the case with vitamin B_{12}, cumulative doses would be intermediate between those for cobalt and those for zero elimination (Table II). Biologic half-lives ranging as high as two years[20,21] have been reported. Although these values of Tb influence relative total radiation exposures, they do not determine the MPC for a labeled vitamin. The maximum permissible concentrations of the several labeled modifications are not known, primarily because the value of f_2 in the human body is unknown for vitamin B_{12}. One may expect the relative MPC's for vitamin B_{12} labeled with the several isotopes to be the same as for elemental cobalt isotopes, since the liver is a target organ. For want of this knowledge for the several modes of administration, and lacking information as to how f_2 varies with dosage level, the administration of vitamin B_{12}-Co^{60} to human subjects has been limited to the 3 μc. dose set for elemental $cobalt^{60}$. In all probability f_2 for vitamin B_{12} is less than 0.68. In one study of a human subject[22] an injection of $Co^{56,57}$-labeled vitamin B_{12} had been given to a patient who died. At autopsy two days later the liver f_2 had a value of $\lesssim$0.3 which would suggest that total body MPC for various isotopic modifications of vitamin B_{12} would be severalfold higher than the values computed for Table II. On the other hand, a value of 0.65 six months after an injection of a 3 μg. dose of vitamin B_{12}-Co^{60} has been reported.[23] Lower fractional liver retention is found in animals; 0.1 to 0.4 in the mouse, hamster and rat.[24,25] The 3 μc. limit on vitamin B_{12} has been applied to the oral administration of vitamin B_{12}-Co^{60}, despite the knowledge that only a fraction of the oral dose is actually absorbed.[26,27] This limit adopted for oral

* Effective half-life is defined[19] as $T = \frac{Tb \times Tr}{Tb + Tr}$ where Tb is the biologic half-life of the element and Tr the radioactivity half-life of the isotope. When the turnover is slow or negligible, as occurs with prolonged retention, $Tb \gg Tr$ and effective half-life is equal to Tr.

† This advantage applies also to handling during synthesis of the vitamin, shipment and storage, since shielding requirements are much less rigorous.

cobalt^{60}-labeled vitamin B_{12}, and the proportionately higher MPC's calculable for the other isotopes, is low by a factor which would depend on the amounts administered. For example, oral absorption of vitamin B_{12} is only $\simeq$40 per cent of a 2 μg. dose and $\simeq$20 per cent of a 5 μg. dose, so that MPC's should be raised accordingly. Currently, however, the MPC's of Table II should be valid although based upon a cobalt instead of a vitamin B_{12} pattern. When sufficient information concerning the distribution of ingested vitamin B_{12} in organs of the human body becomes available, revision of MPC values may be practical.

ADDENDUM

Since delivery of this paper, the National Committee on Radiation Protection has recommended certain changes in exposure limits which have the effect of lowering the permissible exposure to certain parts of the body. These recommendations have been incorporated in Handbook 69 which will replace the current Handbook 52. The U. S. Atomic Energy Commission is also contemplating adoption of these regulations for Commission personnel, contractors and licensees as has been announced in the Federal Register of May 2, 1959. This announcement proposed a new table of maximum body burdens of radioisotopes which now include cobalt^{57} and cobalt^{58}. Total body burdens for cobalt isotopes are, however, listed as 10 μc. for cobalt^{60}, 30 μc. for cobalt^{58} and 200 μc. for cobalt^{57}. This constitutes an increase over MPC's of Handbook 52 of three- to fivefold.

REFERENCES

1. Chaiet, L., Rosenblum, C. and Woodbury, D. T. Biosynthesis of radioactive vitamin B_{12} containing Cobalt-60. *Science*, 3: 601, 1950.
2. Rosenblum, C. and Woodbury, D. T. Cobalt-60 labeled vitamin B_{12} of high specific activity. *Science*, 113: 215, 1951.
3. Smith, E. L., Hockenhull, D. J. D. and Quilter, A. R. J. Tracer studies with B_{12} vitamins. 2. Biosynthesis of vitamin B_{12} labelled with ^{60}Co and ^{32}P. *Biochem. J.*, 52: 387, 1952.
4. Bradley, J. E., Smith, E. L., Baker, S. J. and Mollin, D. L. The use of the radioactive isotope of Cobalt Co^{58} for the preparation of labelled vitamin B_{12}. *Lancet*, 476: 477, 1954.
5. Mollin, D. L. and Smith, E. L. The absorption of vitamin B_{12} and the pathogenesis of vitamin B_{12} deficiency. Proceedings of the International Conference on Peaceful Uses of Atomic Energy, Volume 10, page 475. New York, 1956. United Nations.
6. Strominger, D., Hollander, J. M. and Seaborg, G. T. Table of isotopes. *Rev. Modern Physics*, 30: 585 (1958).
7. U. S. Atomic Energy Commission, Oak Ridge National Laboratory: Radioisotopes, Special Materials and Services (Catalogue and Price List), pp. 80, 81, 154. January, 1957.
8. Gleason, G. I. Abbott Radiopharmaceutical Division, Oak Ridge, Tennessee. Personal communication.
9. Hendlin, D. and Ruger, M. L. The effect of cobalt on the microbial synthesis of LLD active substances. *Science*, 111: 541, 1950.
10. Booth, C. C. and Mollin, D. L. Plasma, tissue and urinary radioactivity after oral administration of ^{56}Co-labelled vitamin B_{12}. *Brit. J. Haemat.*, 2: 223, 1956.
11. Way, K., King, R. W., McGinnis, C. L. and van-Lieshout, R. Nuclear Level Schemes, U. S. Atomic Energy Commission TID-5300, p. 70. Washington, D. C., June 1955.
12. Frauenfelder, H., Levine, N., Rossi, A. and Singer, S. Decay of Co^{58}. *Physical Rev.*, 103: 352, 1956.
13. Cook, C. S. and Tomnovec, F. M. Gamma radiation from Co^{56} and Co^{58}. *Physical Rev.*, 104: 1407, 1956.
14. Booth, C. C. and Mollin, D. L. Plasma, tissue and urinary radioactivity after oral administration of ^{56}Co-labelled vitamin B_{12}. *Brit. J. Haemat.*, 2: 58, 1956.
15. Sakai, M. Angular correlation of gamma rays from Fe^{56}. *J. Phys. Soc. Japan*, 10: 729, 1955.
16. Poppema, O. J., Siekman, J. G., vanWageningen, R. and Tolhoek, H. A. *Physica*, 21: 223, 1955.
17. Bell, P. R. In Siegebahn, K., Beta- and Gamma-Ray Spectroscopy, p. 154. New York, 1955. Interscience Publishers, Inc.
18. U. S. Atomic Energy Commission: Title 10—Atomic Energy, Chapt. 1. Atomic Energy Commission, Part 20. Standards for Protection Against Radiation. *Federal Register*, 22: 548, 1957.
19. U. S. Department of Commerce, National Bureau of Standards, Handbook 52: Maximum Permissible Amounts of Radioisotopes in the Human Body and Maximum Permissible Concentrations in Air and Water. Washington, D. C., March 20, 1953.
20. Schloesser, L. L., Deshpande, P. and Schilling, R. F. Biological turnover of cyanocobalamin (vitamin B_{12}) in human liver. *Arch. Int. Med.*, 101: 306, 1958.
21. Glass, G. B. J. Radioactive vitamin B_{12} in the

liver. III. Hepatic storage and discharge of Co^{60} B_{12} in pernicious anemia. *J. Lab. & Clin. Med.*, 52: 875, 1958.

22. BRUCER, M. Oak Ridge Institute of Nuclear Studies. Personal communication.
23. CRONKITE, E. P., HENLEY, E., DRISCOLL, D. H., MEYER, L. M., DOHAN, F. C., RUBINI, J. R. and WOLINS, W. Studies on the kinetics of intravenously injected ^{60}Cobalt labeled vitamin B_{12} in man. Proceedings of the Seventh International Congress of Hematology, Rome, 1958.
24. ROSENBLUM, C., CHOW, B. F., CONDON, G. P. and YAMAMOTO, R. S. Oral versus parenteral administration of Co^{60}-labeled vitamin B_{12} to rats. *J. Biol. Chem.*, 198: 915, 1952.
25. MILLER, A., GAULL, G., ROSS, J. F. and LEMON, H. M. Tissue distribution of parenteral Co^{60} vitamin B_{12} in mouse, hamster, rat and guinea pig. *Proc. Soc. Exper. Biol. & Med.*, 93: 33, 1956.
26. GLASS, G. B. J., BOYD, L. J. and STEPHANSON, L. Intestinal absorption of vitamin B_{12} in humans as studied by isotope technic. *Proc. Soc. Exper. Biol. & Med.*, 86: 522, 1954.
27. SWENSEID, M. E., GASSTER, M. and HALSTED, J. A. Limits of absorption of orally administered vitamin B_{12}: Effect of intrinsic factor sources. *Proc. Soc. Exper. Biol. & Med.*, 86: 834, 1954.