was oleic acid. In the dog, rat and human, an acid tentatively identified as a C_{22} tetraenoic acid was found in the adrenal CEFA. The significance of these results in relation to hormone synthesis and polyunsaturated fatty acid metabolism is discussed.

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Received May 31, 1960. P.S.E.B.M., 1960, v105.

Plasma Disappearance Rate and Tissue Distribution of Radioactive Cobalt Labelled Cyanocobalamin Injected into Various Animals.* (25991)

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In humans and laboratory animals, parenterally injected cyanocobalamin is rapidly cleared from the blood stream and approaches normal levels within 24-48 hours as determined by microbiological assay methods(1-3). With increased sensitivity attainable with cobalt-60 labelled cyanocobalamin, Miller *et al.* (4) demonstrated that an injected dose of the radioactive vitamin disappeared from the plasma more slowly in patients with chronic myelogenous leukemia than from the plasma of normal subjects. After 24 hours, sufficient radioactivity remained in the plasma to determine the slope of the exponential disappearance curve, which approximated a half life of 5 days. In female rabbits, Rosenthal (5) found that plasma disappearance curves beginning 48 hours after injection of cobalt-60 cyanocobalamin could be resolved into 2 components with apparent half lives of 4.1 days (Component A) and about 50 days (Component B) respectively. The similar plasma disappearance rate of cyanocobalamin in human subjects and rabbits prompted us to determine plasma disappearance rate of injected radio cyanocobalamin in a variety of animal species.

Materials and methods. White rabbits and white leghorn chickens weighing 2-3 kg were obtained from commercial sources. Mongrel dogs weighing 6 to 15 kg were obtained from the local pound. Healthy male human subjects were laboratory personnel weighing 64 to 86 kg. All laboratory animals were maintained on commercial animal feed; water was available *ad lib.* The human subjects ate their usual diets without restriction. The animals were injected with Co^{60}-cyanocobalamin intramuscularly into the left hind leg or intravenously *via* the ear vein in rabbits, wing vein in chickens and jugular vein in dogs. The human subjects were injected with Co^{58}-cyanocobalamin *via* the antecubital vein.

* This study supported by research grant from U.S.P.H.S. I am grateful to Drs. N. S. Ritter, Merck, Sharpe and Dohme for supplies of cobalt-60 labelled cyanocobalamin, to R. J. Dahl, E. R. Squibb and Co., for cobalt-58 Cyanocobalamin, to T. Van Zandt for aid with human studies, and to Paul R. Myers for technical assistance. Some of these data were obtained during author's tenure at Rochester General Hosp., N. Y. An abstract of this study appeared in *Fed. Proc.*, 1960, v10, 416.

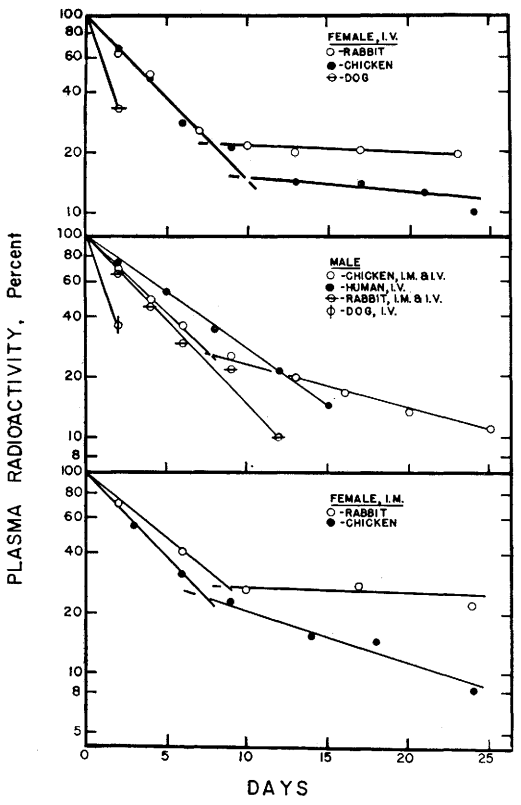

FIG. 1. Disappearance of radioactive cobalt labelled cyanocobalamin from plasma of various animals. Initial values at zero time represent residual radioactivity present in plasma 2 days following inj. of cyanocobalamin. The lines were fitted to experimental values by method of least squares. Each point represents avg values obtained on 3 to 6 animals.

Two days after administration of radioactive vitamin, 8 to 10 ml of blood was obtained for initial estimation of radioactivity by heart puncture from rabbits and chickens, from the jugular vein of dogs and antecubital vein of the human subjects. Similar samples were obtained at periodic intervals. At termination of experiments the rabbits, dogs and chickens were sacrificed and the tissues analyzed for radioactivity by methods previously described (5). Radioactivity in 5 ml samples of plasma was counted in a well type scintillation counter and compared with a standard prepared from the original material at time of injection. Plasma samples were obtained for analysis of radioactivity until the counting rate approached 1.2 times background where the standard error of the count became greater than $\pm$ 10% for a minimum of 10,000 counts. This approximated 3 x 10^{-5} μc per 5 ml of plasma.

Results. The plasma disappearance curves of radio-cyanocobalamin (Fig. 1) were of 2 types, a single component curve in male human subjects and rabbits and a 2 component curve in chickens and female rabbits. The most rapidly disappearing component is designated Component A and the more slowly disappearing one as Component B.

In dogs, plasma radioactivity reached negligible levels within 4 days after injection. Urinary excretion of radioactivity was determined in some dogs and represented less than 2% of injected dose. This value is less than

TABLE I. Plasma Biological Half Life of Radioactive Cyanocobalamin Injected into Various Animals.

Species	Sex	Route	Dose, μc/kg*	Half life $\pm$ S.E. (days) Component A	Component B
Chicken	♀	Intramusc.	.104	4.21 $\pm$ .42 (6)	9.75 $\pm$ 1.01 (6)
	♀	Intrav.	.121	4.06 $\pm$ .34 (6)	31.6 $\pm$ 13.2 (6)
	♂	Intramusc.	.122	4.10 $\pm$ .22 (3)	11.6 $\pm$ .82 (3)
	♂	Intrav.	.140	4.08 $\pm$ .45 (4)	12.5 $\pm$ 1.37 (3)
Rabbit	♀	Intramusc.	.056	4.68 $\pm$ .32 (5)	89.8 $\pm$ 64.5 (3)
	♀	Intrav.	.050	4.34 $\pm$ .29 (6)	38.1 $\pm$ 16.3 (5)
	♂	Intramusc.	.044	3.91 $\pm$ .13 (4)	N.D.
	♂	Intrav.	.046	3.82 $\pm$ .53 (4)	"
		Weighted avg		4.18 $\pm$ .13	29.9 $\pm$ 9.3
Human	♂	Intrav.	.058	5.68 $\pm$ .45 (4)	N.D.
Dog	♀	"	.085	1.34 $\pm$ .27 (4)	"
	♂	"	.069	1.82 $\pm$ .81 (4)	"

* Specific activity of Co^{60} and Co^{58} labelled cyanocobalamin was approximately 1 μc/μg.
No. of animals in parentheses. N.D. = not detected.

urinary excretion of 4% of injected dose previously found in rabbits under similar conditions(5).

The individual disappearance curves were fitted with straight lines by the method of least squares and half life of each component was calculated (Table I). The half life of component A for chickens and rabbits of both sexes was remarkably similar and averaged 4.18 days. The half life of component B, averaging 29.9 days, was significantly longer than that of component A. In male human subjects the half life of component A was 5.68 days. In dogs of both sexes the value was less than 2 days, but this value is of doubtful reliability due to rapid disappearance of the vitamin from the blood stream.

Distribution of radioactivity in tissues of rabbits and chickens 26 days following injection of cobalt-60 cyanocobalamin are shown in Table II. In rabbits, liver and kidney contained the greatest amount in approximately equal quantity, muscle and brain tissue the smallest amount, and heart tissue an intermediate quantity. Distribution of tissue radioactivity appears to be independent of sex or of route of administration of injected vitamin.

In chickens, relative distribution of radioactivity between various tissues was similar to rabbits. Of special interest is the smaller amount of activity present in light meat as compared to dark meat and the similarity between heart and gizzard activity. Specific activity of the liver, kidney, heart and spleen of chickens was usually several times higher than that found in comparable rabbit tissues, but chicken muscle contained less than rabbit muscle.

Tissue content was generally lower in dogs than in rabbits sacrificed 7 days after dosage but relative concentration between tissues was similar for both species with the exception of muscle tissue (Table III). Dog muscle contained approximately 15% of that found in rabbits while other tissues contained about 50%. As with rabbits and chickens, little, if any, differences existed in radioactive content of tissues obtained from male or female dogs.

Discussion. Previous studies have shown

TABLE II. Tissue Radioactivity 26 Days Following Injection of Co^{60} Cyanocobalamin.

Species	Sex	Route	No. animals	Liver	Kidney	Heart	Spleen	Muscle: Dark	Muscle: Light	Gizzard	Brain
Rabbit	♂	Intrav.	4	10.67 ± 1.55	11.85 ± 2.77	4.04 ± .25	3.78 ± .31	1.78 ± .23			2.19 ± .56
	♂	Intramusc.	4	12.19 ± 2.15	15.05 ± 3.99	4.85 ± 1.32	4.31 ± 1.74	3.69 ± .53			2.82 ± .83
	* ♀	Intrav.	5	13.75 ± .59	15.36 ± 1.45	6.73 ± .80	4.41 ± 1.10	2.40 ± .24			2.61 ± .77
	* ♀	Intramusc.	6	13.52 ± 1.70	15.58 ± 2.99	5.79 ± .83	4.82 ± 1.36	2.79 ± .32			
Chicken	♂	Intrav.	3	56.47 ± 9.02	64.63 ± 10.50	6.38 ± 4.08	9.49 ± .57	1.26 ± .08	.42 ± .04	5.19 ± .54	
	♂	Intramusc.	4	46.04 ± 3.18	67.42 ± 14.24	12.69 ± 1.93	11.32 ± .44	1.36 ± .20	.63 ± .21	4.08 ± .66	
	♀	Intrav.	3	84.15 ± 5.59	86.67 ± 26.81	22.82 ± 1.97	18.62 ± 2.82	1.64 ± .17	.52 ± .09	6.99 ± 2.70	
	♀	Intramusc.	4	82.08 ± 7.64	68.39 ± 10.90	17.85 ± 1.05	14.56 ± 1.88	1.39 ± .19	.86 ± .09	6.34 ± 1.12	

* From Rosenthal, 1959(5).

Values in terms of percentage of dose/100 g tissue ± stand. error.

TABLE III. Tissue Radioactivity 7 Days Following Injection of Co^{60} Cyanocobalamin.

Species	Sex	Route	No. animals	Liver	Kidney	Heart	Spleen	Muscle	Brain
Dog	♂	Intrav.	4	4.13 ± .41	5.32 ± .48	2.32 ±.62	6.42 ± .22	.44 ±.09	1.19 ±.22
	♀	"	4	5.96 ± .84	8.25 ± .36	3.85 ±.79	8.77 ±1.88	.59 ±.08	1.61 ±.13
Rabbit*	♀	Intramusc.	6	13.28 ±1.16	12.67 ±1.62	7.16 ±.27	10.80 ±1.99	3.56 ±.11	2.80 ±.59

* From Rosenthal, 1959(5).

Values in terms of percentage of dose/100 g of tissue ± stand. error.

that plasma level and tissue concentration of Vit. B_{12} varies widely between different species of animals(6-8). It is somewhat surprising therefore, to find that the apparent disappearance rate of component A in plasma is so remarkably similar in animals as diverse as rabbits, chickens and human subjects. The different half life of component A for human subjects (5.7 days) as compared with chickens and rabbits (4.2 days) may be due to experimental error since, in the smaller animals, withdrawal of sufficient blood to yield 5 ml of plasma for radioactivity determinations represents a significant proportion of the blood volume with concomitant removal of the bound vitamin. This tends to decrease the value of the observed half life. In contrast to the laboratory animals, withdrawal of similar quantities of blood from human subjects represents a small percentage of blood volume. If one estimates a correction for removal of radioactivity, the half life of component A for chickens and rabbits becomes 5.3 days, a value consistent with that for male human subjects. The 5.7 day half life for component A in normal human subjects reported here is surprisingly similar to the 5 day half life found in sera of patients with chronic myelogenous leukemia determined by Miller *et al.*(4). Furthermore, Reizenstein(9) has recently calculated the apparent biological half life of plasma Vit. B_{12} in human subjects to be 6 days, a value in complete agreement with our experimental data.

The finding that 2 well defined disappearance curves are present in plasma of female rabbits and only one in male rabbits, suggests the possibility of an endocrine relationship. On the other hand, no sex relationship is apparent in chickens.

Disappearance rates shown in Fig. 1 and Table I have not been corrected for disappearance rate of component B. It must be recognized that disappearance rate of component A may be modified by the slower disappearance rate of component B. Until further information is available, it appears desirable to present our data in its actual uncorrected form.

Although Vit. B_{12} content of serum varies widely between various species of animals, the similarity of disappearance rate of component A indicates that the protein-cyanocobalamin complex in various animals may be qualitatively similar. On the other hand, Rosenthal(10) showed that disappearance rate of component A in rabbits given oral doses of Co^{60} cyanocobalamin was slower ($T\frac{1}{2} = 7.1$ days) than parenterally injected vitamin. These data demonstrate qualitative differences in binding affinities depending on route of administration.

The wide variation of radioactivity in tissues of various species demonstrates that comparable tissues from different animals have different Vit. B_{12} binding affinities. This is evident by comparing tissues of chickens and dogs with that of rabbits. Reasons for this wide variation are unknown and must await further clarification.

Summary. Plasma disappearance of parenterally injected radio cyanocobalamin in various animals was determined. Beginning 48 hours after injection 2 disappearance curves were found in female rabbits and chickens of both sexes, but only 1 disappearance curve was present in male rabbits and male human subjects. The most rapidly disappearing component (Component A) was similar in rabbits and chickens ($T\frac{1}{2} = 4.2$ days) and human subjects ($T\frac{1}{2} = 5.7$ days). The more slowly disappearing component (Component B) in female rabbits and chick-

ens of both sexes approximated a biological half life of 29.9 days. In dogs of both sexes, plasma disappearance rate was very rapid (T ½ = 1.5 days). Tissue radioactivity varied between tissues of the same species and between the various species studied.

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Received June 3, 1960. P.S.E.B.M., 1960, v105.

Isolation and Elimination of Pleuropneumonia-Like Organisms From Mammalian Cell Cultures.* (25992)

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Following the report of Robinson, *et al.*(1) on association of pleuropneumonia-like organisms (PPLO) with human cells in culture, high incidence of PPLO contamination has been found in several laboratories(2-4). Our findings have been briefly presented in preliminary communications(5,6). Methods for surveillance of animal cell cultures for PPLO and for their elimination are important to application of cell cultures in virological and physiological research, since PPLO contamination is not revealed by grossly visible changes in culture appearance or cellular morphology. Neither are influences of PPLO on metabolism of animal cells in culture known. This paper reports results of a program instituted in the Dept. of Bacteriology, Univ. of Minnesota, for isolation and cultivation of PPLO from established animal cell lines. Use of the antibiotic kanamycin for elimination of PPLO from contaminated cell lines is described.

Materials and methods. Cells tested were mostly those propagated in this laboratory in continuous culture for varying periods from 2 to 5 years. Twenty-four lines submitted by other investigators were also tested. Since results were communicated individually to submitting investigators, these results are combined with other data for presentation here. Fifteen lines, all but one propagated by the same worker for more than 2 years, were used for evaluation of the effectiveness of kanamycin. Cell lines from Minnesota were propagated as described(7), while other cells were cultivated in routinely employed media. All media contained human, horse, calf or rabbit serum supplemented with a) yeast extract in Hanks' balanced salt solution or b) Eagle basal medium(8). Serum pools were Selas-filtered and inactivated at 56°C for 30 minutes before use. *Trypsin* (Difco 1:250) in 10 times stock solution was prepared by dissolving 0.2 g in 100 ml of GKN solution (5.5 mM glucose, 5.4 mM potassium chloride, 138 mM sodium chloride). Trypsin stock was adjusted to pH 7.8 with sodium bicarbonate (166 mM) solution, sterilized by Selas filtration, and stored at –20°C. *Kanamycin sulfate*† was dissolved in Hanks' balanced salt solution to a concentration of 10^4 μg kanamycin base per ml.

PPLO medium, modified from the formulae

* Aided by grants from Am. Cancer Soc., Nat. Foundation and Nat. Cancer Inst., Nat. Inst. Health, P.H.S.

† Kanamycin sulfate was supplied kindly by Dr. C. H. Mann, Bristol Labs., Inc.