

VITAMIN B₁₂Co⁶⁰ READILY PASSES THE PLACENTA INTO
FETAL ORGANS AND NURSING PROVIDES B₁₂ FROM
MOTHER TO PUP*, †, §

A RECORD OF ITS DISTRIBUTION

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During the past six years, the distribution of radioactive B₁₂Co⁶⁰ in dog organs and tissues has been studied in this laboratory. Many unexpected findings have been reported but not explained (13, 14). High concentrations of radioactive B₁₂ in heart muscle, gastric mucosa, liver, and brain were reported in long term experiments. Obviously, this B₁₂ is moved about according to need, as would happen with a physiologically active substance. In anemia, the new red cell stroma may be rich in B₁₂Co⁶⁰, whereas in normal periods there is little or no radioactive B₁₂ in red cells or bone marrow (13).

The experiments tabulated below show clearly that the B₁₂Co⁶⁰ passes the placental barrier with ease, and also that nursing supplies this material from the mother, and they enable us to study the distribution of B₁₂ in the pup's tissues after nursing. Here, too, with passage of time, there is a movement of radioactive B₁₂ from some storage areas to other organs, much as is observed in long time experiments in adult dogs. Nursing causes a drain on the mother's store of vitamin B₁₂.

Adult dogs poisoned with chloroform, much to our surprise, showed no significant change in liver concentration of B₁₂Co⁶⁰, in spite of severe chloroform injury of the hepatic cells. An adult dog injured by a calculated, fatal, x-ray exposure, likewise showed no significant change in organ distribution of B₁₂.

The evidence supports the belief that B₁₂Co⁶⁰ remains in the dogs' tissues over long periods of time as a physiologically active substance. It behaves differently from inorganic Co⁶⁰ (12).

Cooperman *et al.* (4) found that the radioactive vitamin distributed itself in all of the viscera like natural B₁₂, and they consider that B₁₂Co⁶⁰ is physiologically active.

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§ The Vitamin B₁₂ labeled with radioactive cobalt was received from Merck and Company.

Glass and Mersheimer (5) demonstrated that the radioactive material extracted from liver is microbiologically active. Reizenstein (10), in a review article of vitamin B₁₂ metabolism, considers that the radioactive vitamin is present as a physiological vitamin; and he also postulates an enterohepatic circulation of B₁₂ with possibly some secretion of it by the pancreas. Grasbeck *et al.* (6) also conclude that there is an enterohepatic circulation of B₁₂.

Numerous reports have been written indicating the passage of B₁₂Co⁶⁰ through the placenta (8, 5, 11, 3), but none has gone into the extensive detail of placental and nursing transfer with the final distribution of the radioactive material in the pups.

TABLE 1
Placenta Transfer of Radioactive B₁₂ to the Organs of Pups during 8 Days
(All pups less than 12 hours old—Dog 57-372)
Autopsy counts per minute per gram wet weight

Pup.....	A	B	C	D	E
Heart.....	761	511	588	771	1045
Lung.....	960	1249	790	982	1465
Liver.....	1082	800	1091	1432	1370
Spleen.....	4045	4880	1652	2311	4140
Pancreas.....	6130		4945	4430	5181
Kidney.....	1378	2185	1522	1768	2075
Brain.....	1175	1234	770	909	969
Stomach.....	1460	1900	1512	1260	2260
Small intestine.....	2669	3160	1263	1970	2181
Thymus.....	3140	3081	2111	2579	3165
Diaphragm.....	528	332	199		450
Placenta.....	1549	1769	2042		

Dog 57-372. November 18, 1957, injected B₁₂Co⁶⁰ (counts per minute—7,984,800); November 18, 1957, delivery; nursing 0 to less than 12 hours.

In studies of human beings, it has been demonstrated that serum B₁₂ levels are low in the mother with increased amounts in the fetus (2, 7). Nesbitt and Chow (9), in a review article, discuss the needs for B₁₂ in pregnancy in human beings.

Methods

All the animals were sacrificed with ether anesthesia or by intravenous injection of nembutal; and organs, tissues, blood, bile, and urine were saved and sampled for Co⁶⁰ activity. For measurements of radioactivity, 1 gm. portions, or the whole organ (when the weight was less than 1 gm.), or 1 cc., in the case of fluid, were put in 13 × 100 ml. pyrex culture tubes and placed in a well type scintillation counter, similar to that described by Anger (1). The net weight of the organ sample was determined by subtracting weight of tube from weight of organ plus tube. Measurements are expressed as counts per minute per gram of wet tissue. Standards were made on each sample of vitamin B₁₂Co⁶⁰ in order to determine actual number of counts injected. The vitamin was injected subcutaneously; and the dogs were kept in metabolism cages, with urine collected and analyzed for radioactive material excreted during several days following injection. The vitamin B₁₂, supplied by Merck and Company,

Inc., contained on an average 21 micrograms of labeled vitamin per 20 cc. vial. Each dog was given 9 to 10 cc. of this material subcutaneously.

EXPERIMENTAL OBSERVATIONS

Our first trial experiments showed that radioactive B₁₂ passed promptly through the placenta. In as short a time as 17 hours after injection of the moth-

TABLE 2
Total Organ Counts per Minute of Radioactive B₁₂ and Organ Weight of the Pups
at Autopsy
(Dog 57-372 litter mates)

Pup.....	A	B	C	D	E
Heart, counts.....	2,055	1,635	1,411	1,310	3,762
gm.....	2.7	3.2	2.4	1.7	3.6
Lungs, counts.....	7,392	5,496	7,189	6,481	12,306
gm.....	7.7	4.4	9.1	6.6	8.4
Liver, counts.....	22,722	20,000	31,639	31,504	26,030
gm.....	21.0	25.0	29.0	22.0	19.0
Spleen, counts.....	1,679	2,377	1,008	1,029	3,062
gm.....	0.40	0.49	0.61	0.45	0.75
Pancreas, counts.....	3,079		2,721	1,812	4,867
gm.....	0.50		0.55	0.41	0.94
Kidney, counts.....	5,650	9,177	7,153	6,011	9,338
gm.....	4.1	4.2	4.7	3.4	4.5
Brain, counts.....	7,638				
gm.....	6.5				
Thymus, counts.....	1,677	1,749	1,639	1,653	2,556
gm.....	0.53	0.56	0.77	0.64	0.81
Placenta counts.....	55,764	62,529	110,268		
gm.....	36.0	41.0	54.0		

ers, the placentas were found to be very radioactive; and the pup organs showed small amounts of B₁₂ activity. This led us to perform the following detailed studies relating to placental and nursing transfer of B₁₂Co⁶⁰.

Dog 57-372 started delivery during the night 8 days following the subcutaneous injection of B₁₂Co⁶⁰. However, two pups were born after 9 a.m. so that two placentas were obtained had another one was recovered from the stomach of the mother at autopsy. The two pups had no opportunity to nurse and the other three probably nursed for less than 12 hours.

Table 1 illustrates the range of counts per gram of wet weight, and it is evident that all the organs contained radioactive material as the result of transfer from the mother through the placenta. The activity in the small intestines of these new-born pups was high, approaching that of the stomach, and this is in

TABLE 3
Placental Transfer and Nursing Transfer of Radioactive B₁₂
(Dog 59-260 litter mates)
Autopsy counts per minute per gram wet weight

Pup—Age, <i>days</i>	1	14	22	24
Weight, <i>gm.</i>	339	844	540	576
Heart	620	356	595	577
Lung	765	298	258	495
Liver	848	485	798	1057
Spleen	1705	331	700	884
Pancreas	1724	855	1206	1300
Stomach	621	474	373	1026
Small intestine	556	205	206	278
Colon	598	206	158	239
Kidney	907	550	708	805
Brain	1005	268	423	437
Spinal cord	1014	334	464	443
Pituitary		38*	18*	50*
Thymus	2117	530	1098	1200
Thyroid	504	366	542	480
Adrenal	1280	636	793	866
Bladder	247	131	227	323
Submaxillary	393	289	460	405
Lymph nodes	1042	299		
Uterus	416		437	280
Ovaries or testicles	1499	269	733	713
Diaphragm	200	215	318	374
Striated muscle	230	133	294	328

* Actual counts for total gland.

Dog 59-260. May 15, 1959, injected B₁₂Co⁶⁰ (counts per minute—7,355,000); May 24, 1959, delivery.

contrast to the much lower counts in the small intestines of older pups. Table 2 gives the total counts and organ weights, and demonstrates clearly there was considerable radioactivity. These data will serve as a base line when comparing the results with those of increased radioactivity due to nursing.

Dog 59-260 appeared active and healthy; but, a few hours after the injection of the vitamin, it was observed to have some exudate about the nostrils. Distemper was suspected but the dog never appeared seriously ill; and, 9 days after her injection, six healthy pups were born. One pup was killed at the end of the 1st day as control to learn the amount of radioactivity in its tissues as result of placental transfer. Fourteen days later, a second pup was examined.

It appeared healthy and had gained weight. The last two pups developed exudate on their conjunctivae, were sick, and had not gained weight, as indicated in the table.

Although this experiment is complicated by infection, the data are still of value and again show the transfer of vitamin by way of the placenta. All tis-

TABLE 4
Total Organ Counts per Minute of Radioactive B₁₂ and Organ Weight of Pups at Autopsy
(Dog 59-260 litter mates)

Pup—Age, days	1	14	22	24
Heart, counts.....	2,170	2,007	2,201	3,058
gm.....	3.5	5.6	3.7	5.3
Lungs, counts.....	5,580	3,874	1,720	3,316
gm.....	7.3	13.0	6.7	6.7
Liver, counts.....	14,210	21,582	28,500	43,971
gm.....	16.8	44.5	35.7	41.6
Spleen, counts.....	1,444	1,456	775	1,592
gm.....	0.85	4.4	1.1	1.8
Pancreas, counts.....	1,741	2,137	1,615	2,281
gm.....	1.0	2.5	1.3	1.8
Kidney, counts.....	1,360	3,052	2,987	4,508
gm.....	1.5	5.6	4.2	5.6
Brain, counts.....	8,361	6,566	10,359	13,328
gm.....	8.3	24.5	24.5	30.5
Stomach, counts.....	950	3,033	2,323	5,643
gm.....	1.5	6.4	6.2	5.5
Striated muscle, counts.....	41,970	42,560	60,270	88,290
gm.....	139.0	320.0	205.0	218.0
Thymus, counts.....	1,925	1,817	414	817
gm.....	0.91	3.4	0.38	0.68
Thyroid, counts.....	54	67	93	85
gm.....	0.11	0.18	0.17	0.18
Adrenals, counts.....	102	126	211	130
gm.....	0.08	0.20	0.27	0.15
Pituitary, counts.....		38	18	50
gm.....		0.01	0.006	0.02

sues contain radioactive B₁₂, as illustrated in Table 3. On review of the total organ counts, as given in Table 4, it is evident that the three pups which nursed had a higher content of labeled vitamin than pup 1. There was progressive increase, particularly in the heart, liver, kidney, brain, and stomach; and

TABLE 5
Placental Transfer and Nursing Transfer of Radioactive B₁₂
Autopsy counts per minute per gram wet weight
(Dog 58-293 litter mates)

Pup—Age, days	2	2	9	23	43	155
Weight, kg.	(runt)			1.1	3.5	11.0
Heart	116	296	485	352	272	131
Lung	107	515	356	324	118	17
Liver	516	1089	1189	453	490	49
Spleen	464	2800	996	560	244	32
Pancreas	865	1030	1589	826	524	46
Stomach	238	486	714	622	647	102
Small intestine	273	990	244	310	63	14
Colon	113	370	436	250	70	6
Kidney	172	616	808	602	491	96
Brain	124	300	328	251	206	123
Spinal cord					153	20
Pituitary					36*	10*
Thymus	679	730	1655	336	161	13
Thyroid	1629	1182	1076	642	183	17
Adrenal	267	2531	1660	772	563	17
Bladder		203	423	107	58	41
Submaxillary	125	268	378	364	178	4
Lymph nodes					207	8
Uterus	112				148	
Ovaries or testicles	584	857	834	441	482	58
Diaphragm		192	219	238	197	34
Striated muscle	45	156	430	129	86	18

* Actual counts for total gland.

Dog 58-293, May 19, 1959, injected B₁₂Co⁶⁰ (counts per minute—5,958,000); May 24, 1959, delivery.

this augmentation must have been the result of nursing. The values for striated skeletal muscle are estimated on the basis that muscle comprises 38 per cent of body weight.

Tables 5 and 6 (Dog 58-293) give data on a satisfactory experiment. Ten pups were born 5 days after injection of the mother and they were all healthy, but one was a runt. The radioactivity of the tissues of this runt was definitely less in amount, than that of a normal-sized pup examined at the same time. A 43-day-old pup showed the maximum content of active B₁₂. By this time,

it had had a complete period of nursing and was also eating food. Subsequent pups with increasing age and size of organs show a definite decrease in B₁₂ on per gram basis (Table 5). Total counts per organ (Table 6) also show decrease, except for the heart and brain which continue to show the same high level. This decrease may be partly due to loss of the vitamin and dilution of it as organs increase in size. There may well be some shifting of the vitamin from some of

TABLE 6
Total Organ Counts per Minute of Radioactive B₁₂ and Organ Weight of Pups at Autopsy
(Dog 58-293 litter mates)

Pup—Age, days Weight, kg.	2 (runt)	2	9	23 1.1	43 3.5	155 11.0
Heart, counts	163	651	970	2,675	6,256	9,170
gm.	1.4	2.2	2.0	7.6	23.0	70.0
Lungs, counts	214	2,575	1,851	5,443	4,649	1,599
gm.	2.0	5.0	5.2	16.8	39.9	97.0
Liver, counts	3,199	13,939	14,387	26,048	68,110	17,200
gm.	6.2	12.8	12.1	57.5	139.0	352.0
Spleen, counts	106	1,976	1,422	3,360	3,026	833
gm.	0.23	0.71	1.4	6.0	12.4	26.0
Pancreas, counts	236	1,087	1,093	2,726	6,183	1,076
gm.	0.27	1.1	0.69	3.3	11.8	23.5
Kidney, counts	206	1,848	2,747	4,214	6,874	2,400
gm.	1.2	3.0	3.4	7.0	14.0	25.0
Brain, counts	694	1,980	3,083	7,781	12,030	11,034
gm.	5.6	6.6	9.4	31.0	58.4	90.0
Stomach, counts	169	645	612	6,344	27,174	14,650
gm.	0.71	1.33	0.86	10.2	42.0	145.0

the organs to maintain the high levels of the heart and brain. This shifting about is similar to that which we have observed previously in long term experiments in normal adult dogs. It is interesting to note that, after 155 days, all the organs still retained considerable activity.

Tables 7 and 8 give data on another satisfactory experiment.

Dog 59-261 gave birth to seven pups 2 days after receiving the injection of B₁₂Co⁶⁰. They were all healthy and continued so. The control pup 1 shows activity in its organs as the result of placental transfer. There is increasing

activity as the result of nursing on a per gram basis (Table 7) up to the 58th day; and then, with cessation of nursing, decreasing activity is apparent. In Table 8, in which total counts per organ are considered, the increased activity is very apparent, and, also, the subsequent decrease after the 58th day; although,

TABLE 7
Placental Transfer and Nursing Transfer of Radioactive B₁₂
Autopsy counts per minute per gram wet weight
(Dog 59-261 litter mates)

Pup—Age, days..... Weight, gm.....	1 243	15 462	22 648	58 1300	106 2000	127 4000	177 7300
Heart.....	68	810	810	626	369	298	127
Lung.....	241	783	529	216	7	23	26
Liver.....	125	1722	1408	1040	260	152	64
Spleen.....	271	1900	1050	340	611	77	26
Pancreas.....	630	2146	779	1010	276	133	
Stomach.....	244	1134	987	856	188	155	64
Small intestine.....	2100	606	437	137	57	34	26
Colon.....	124	554	362	133	55	35	10
Kidney.....	199	1358	1107	798	1355	233	169
Brain.....	63	686	354	360	249	208	187
Spinal cord.....	218	700	466	233	78	68	48
Pituitary.....	81*	20*	60*	20*	21*	10*	
Thymus.....	135	1006	895	324	75	29	15
Thyroid.....	148	1135	817	535	131	61	17
Adrenal.....	35	1942	2000	732	282	89	48
Bladder.....	40	290	318	250	91		35
Submaxillary.....	50	684	550	424	193	114	46
Lymph nodes.....	245	744	596	162	68	47	35
Uterus.....		536		318			6
Ovaries or testicles.....	58	1130	419	440	119	61	19
Diaphragm.....	37	435	468	351	196	105	
Striated muscle.....	20	350	218	197	102	46	67

* Actual counts for total gland.

Dog 59-261, May 23, 1959, injected B₁₂Co⁶⁰ (counts per minute—6,913,700); May 25, 1959, delivery.

again, the brain and heart tend to retain higher levels of vitamin. Dilution of vitamin as the result of increased size of organ, again, may be a factor; but, also, shifting may have occurred. The long persistence of B₁₂Co⁶⁰ in organs such as the heart, liver, stomach, kidneys, brain, and muscle indicates that it serves a physiological purpose and is not an inert fraction.

For completeness, we examined the organs and tissues of the adult mother dogs sacrificed, as indicated, days or months after injection of the vitamin

(Tables 9 and 10). Dog 57-372 was killed after delivery 7 days following vitamin injection. Its organs were all very radioactive, as would be expected in such an acute experiment. The uterus was particularly active, as were the placentas.

TABLE 8
Total Organ Counts per Minute of Radioactive B₁₂ and Organ Weight of Pups at Autopsy
(Dog 59-261 Litter Mates)

Pup—Age, days Weight, gm.	1 243	15 462	22 648	58 1300	106 2000	127 4000	177 7300
Heart, counts	182	2,592	4,536	5,914	5,535	10,430	5,300
gm.	2.67	3.2	5.6	9.4	15.0	35.0	41.9
Lungs, counts	1,055	5,481	5,131	3,024	314	1,495	1,941
gm.	4.4	7.0	9.7	14.0	49.0	65.0	74.6
Liver, counts	1,650	39,606	47,592	79,976	23,360	31,920	15,360
gm.	13.2	23.0	39.3	76.9	91.0	210.0	240.0
Spleen, counts	271	6,270	4,095	1,163	611	501	411
gm.	0.87	3.3	3.9	3.4	4.6	6.5	15.8
Pancreas, counts	630	2,167	1,636	4,949	1,904	1,583	
gm.	0.52	1.1	2.1	4.9	6.9	11.9	
Kidney, counts	334	1,358	5,535	4,221		3,550	1,286
gm.	1.7	3.9	5.0	6.3		25.0	17.4
Brain, counts	448	14,508	10,939	18,828	17,928	15,475	14,125
gm.	7.1	21.1	30.9	52.3	72.0	74.4	75.6
Stomach, counts	439	3,515	7,205	18,233	9,400	10,710	7,040
gm.	1.8	3.1	7.3	21.3	50.0	70.0	80.0
Skeletal muscle, counts	1,840	61,600	49,628	97,318	77,520	69,920	185,858
gm.	92.0	176.0	246.0	494.0	760.0	1520.0	2774.0
Thymus, counts	83	2,032	1,969	810	196	290	263
gm.	0.62	2.0	2.2	2.5	2.6	10.0	18.1
Thyroid, counts	8	168	130	113	48	43	11
gm.	0.05	0.15	0.16	0.21	0.37	0.71	0.64
Adrenal, counts	2	332	254	308	146	42	37
gm.	0.06	0.17	0.13	0.42	0.52	0.38	0.77

In contrast with long term experiments, the spleen, pancreas, kidneys were very active, indicating that, at first, all tissues tend to accumulate the B₁₂Co⁶⁰.

Dog 58-517 served as a control in a long term experiment of 186 days. Ten days prior to death, it received 600 roentgens total body radiation, a calculated fatal dose. This radiation did not appear to alter the distribution of vitamin B₁₂; and we see very marked persisting activity, particularly in the heart, liver, pancreas, kidneys, stomach, and brain.

Dogs 59-260, 59-261, and 58-293 were pregnant dogs that were allowed to live following delivery so they could nurse their pups. There was a very definite decrease in active B₁₂ material in all the tissues and organs on a per gram basis and, also, on total organ counts (Table 9 and 10). It would appear that nursing had depleted the stores of vitamin.

TABLE 9
Autopsy Counts per Minute of Radioactive B₁₂ per Gram Wet Weight of the Organs of Adult Dogs

Dog	57-372	59-260	59-261	58-293	58-517
Days after injection.....	7	167	207	225	186
Heart.....	1065	1150	934	524	1797
Lung.....	384	122	106	60	163
Liver.....	2740	318	356	287	878
Spleen.....	2961	268	217	180	458
Pancreas.....	5619	574	345	475	1550
Stomach.....	9760	1035	797	584	2523
Small intestine.....	1911	91	143	56	203
Colon.....	1140	52	118	39	133
Kidney.....	4301	654	408	378	1605
Brain.....	814	380		334	568
Spinal cord.....		148		104	214
Pituitary.....	713*	19*		73*	407*
Thyroid.....	966	99		160	183
Adrenal.....	3235	540	353	285	1047
Bladder.....	320	132		60	213
Breast tissue.....	7540				
Submaxillary.....	800	327		204	809
Lymph nodes.....	1634	160		43	111
Uterus.....	2122	132	75	95	128
Ovaries.....	6570	30	134	86	
Diaphragm.....		266	152	229	244
Striated muscle.....	219	184		97	139

* Actual counts for total gland.

Dogs 59-260 and 59-261 were used in experiments with chloroform to determine whether liver damage would result in mobilization of vitamin B₁₂ from the liver. They were fasted for 3 days and then given 1 hour of CHCl₃ anesthesia. Dog 59-260 had very severe liver injury, as determined by degree of icterus and the clinical picture, but recovered and was killed on the 10th day. The injury to the liver was almost completely repaired, as shown by histological examination. Serial urine counts for B₁₂Co⁶⁰ were negative. Dog 59-261 was killed after 48 hours at the height of the liver injury. There was a decreased amount of vitamin B₁₂ in the livers, but the liver of normal pregnant dog 58-293

showed an even lower concentration. Therefore, we cannot state that any conspicuous mobilization was caused by the liver necrosis. Additional studies are needed before conclusions can be drawn.

TABLE 10
Total Organ Counts of Radioactive B₁₂ and Organ Weight of Adult Dogs at Autopsy

Dog	57-372	59-260	59-261	58-293	58-517
Days after injection	7	167	207	225	186
Weight, kg.....		14.0		11.5	
Heart, counts.....	95,850	138,000	74,790	21,484	161,730
gm.....	90.	120.	80.	41.	90.
Lung, counts.....	38,400	6,100	7,685	5,296	13,855
gm.....	100.	110	73.	89.	85.
Liver, counts.....	1,117,920	96,000	121,500	68,109	245,840
gm.....	408.	320.	300.	307.	280.
Spleen, counts.....	71,064	12,060	4,774	3,600	9,160
gm.....	24.	45.	22.	20.	20.
Pancreas, counts.....	185,427	17,220	6,865	14,725	46,500
gm.....	33.	30.	17.	31.	30.
Kidney, counts.....	227,953	26,160	7,980	23,814	32,100
gm.....	53.	40.	21.	63.	20.
Brain, counts.....	54,538	26,600		23,714	39,760
gm.....	67.	70.		71.	70.
Stomach, counts.....		124,200	63,760	55,772	336,500
gm.....		120.	80.	95.	100.
Skeletal muscle, counts.....		988,880		423,890	
gm.....		5,320.		4,370.	
Uterus, counts.....	345,886				
gm.....	163.				
Injected B ₁₂ Co ⁶⁰ Counts.....	7,984,000	7,355,000	6,914,000	5,958,000	7,435,000
	Pregnancy	Pregnancy Later CHCl ₃	Pregnancy Later CHCl ₃	Pregnancy	Non-pregnant X-ray

In all of the puppies, samples of skin, skull, and vertebrae were analyzed; and they show the presence of active B₁₂, although not in high concentrations. The bile was also counted with counts ranging from 27 to 300 per cc. noted in older pups, from which it would appear that some vitamin is continuously being excreted in the bile. Some of the pups had either round worms or tape worms, and it was found that these worms had an activity ranging from 16 to 3400 counts per gram. The highest activity was generally in the worms from the pups which were still nursing, with a marked decrease in non-nursing

pups. This would indicate direct absorption of the vitamin from the intestinal contents.

DISCUSSION

Brain tissue in dogs and pups contains high concentrations of B₁₂. This increase within brain tissue goes on slowly but uniformly (see tables for ages). The brain contains more B₁₂ per gram than does the spinal cord. It is well known that, in pernicious anemia due to lack of B₁₂, there may develop serious central nervous system abnormalities which, if not too far advanced, can be cleared up by B₁₂ therapy. All this suggests that B₁₂ is a constant component of brain tissue, obviously of functional importance, and that the turnover is slow. How much damage could this radioactive B₁₂ cause in the brain tissue? We have no evidence that actual damage is done but must admit such a possibility with time.

Heart muscle in fetus, pup, and dog contains a rather high content of B₁₂ per gram and this certainly suggests a functional activity, as the heart muscle does not store inert material. Compared with heart muscle, the striated muscle of the body contains only 10 to 20 per cent as much per gram. The diaphragm contains more B₁₂ than the striated muscle of the body and legs, perhaps, we may say, as a result of its more and regular activity. Smooth muscle is lower in B₁₂ content than skeletal muscle. This suggests that B₁₂, in some way, may take a part in energy production.

Gastric mucosa from the body of the stomach contains B₁₂CO⁶⁰ in rather high content per gram, which closely approximates that found in the heart muscle in pups and adults. The adults were given the radioactive B₁₂ subcutaneously and hence the amounts found in the gastric mucosa must have come through the blood stream. The older pups gained most of the B₁₂ by nursing, and one might say that the deposit came directly from the gastric content; but it seems more likely to have been absorbed by the intestinal tract and deposited by the blood stream in the gastric mucosa. Elsewhere, the mature intestinal mucosa showed low levels of B₁₂. The significance of this peculiar localization of B₁₂ in the gastric mucosa surely deserves further study. Observations on human beings given B₁₂CO⁶⁰, with subsequent autopsy analysis, would be of great value. An explanation of Castle's intrinsic factor might be forthcoming.

The small and large intestinal mucosa contain but small amounts of B₁₂CO⁶⁰, when measured in adult dogs. When a pregnant dog is given B₁₂CO⁶⁰ some days before delivery, we observe that pups only 1 or 2 days old may show as much B₁₂ in the small and large intestinal mucosa as in the gastric mucosa. As the pups nurse and grow larger, the gastric mucosa remains high in B₁₂ while the small and large intestinal mucosa show low values of B₁₂, much as in the adult dog. No convincing explanation of these differences has been given.

The pancreas in pups and adults shows rather high content of B_{12} , while the submaxillary gland rates much lower. It is at least possible that the pancreas may secrete B_{12} , but no such evidence is recorded.

We know that radioactive material does appear in small amounts in the bile. This probably is $B_{12}Co^{60}$, but it may be some split product. We have observed that radioactivity can be abundant in intestinal parasites (round and tape worms) in older nursing pups and adult dogs. This is probably $B_{12}Co^{60}$, and the fact suggests that the intestinal lumen may contain significant amounts of radioactive B_{12} . In some cases, it probably means from the milk of radioactive mothers; but, again, it may mean B_{12} from the bile or pancreas. Does this imply a circulation of B_{12} within the body (liver or pancreas) of the pup or adult? There is at least a possibility that the liver or pancreas, or both, may secrete $B_{12}Co^{60}$ into the intestine, with its subsequent absorption.

The kidney contains a considerable amount of radioactive B_{12} in dogs and pups; and B_{12} is secreted in the urine, especially during the 1st few days in adult dogs after injection with $B_{12}Co^{60}$. It is at least possible that some of the B_{12} may be re-absorbed by the kidney tubules and returned to the body circulation.

The spleen shows high counts in adults and pups shortly after introduction of the $B_{12}Co^{60}$, whether it is given subcutaneously in adults or passed through the placenta to the pups. With the passage of time, after weeks or months, the concentration of $B_{12}Co^{60}$ in the spleen decreases as the distribution of B_{12} goes on within the body. This shows a course of events that is the opposite of that in the brain. As the concentration within the spleen decreases, the brain content increases. Evidently, the spleen is a temporary storehouse of an excess of B_{12} which subsequently is redistributed.

SUMMARY

The placenta is permeable to $B_{12}Co^{60}$. Together with salts, sugar, amino acids, vitamins, and proteins, the B_{12} is contributed to the growing embryos from the mother's body stores. At birth, the placenta contains a liberal amount of B_{12} , which the mother regains by ingestion of the placentas.

Nursing draws a liberal amount of $B_{12}Co^{60}$ from the mother's stores and contributes it to the body of the pups where it is absorbed and distributed in the various organs, much as noted when adult dogs are injected with $B_{12}Co^{60}$. A redistribution is noted as the months pass; but the high values in the heart, liver, and gastric mucosa persist, and the brain usually shows a slow increase.

Enterohepatic circulation of B_{12} may involve bile and enteric content plus hepatic, gastric, and pancreatic epithelial secretion. This type of body recycling of radioactive B_{12} is discussed but not proven.

High values of B_{12} in the heart, brain, gastric mucosa, and liver indicate that the vitamin is functionally active, not an inert fraction.

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