The Distribution of Radioactive Cobalt in Pigs

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The discovery (Smith, 1948; Rickes, Brink, Koniuszy, Wood & Folkers, 1948) that vitamin B_{12} contains cobalt immediately suggested that it might be possible to label the molecule with radioactive cobalt. A radioactive form of the vitamin would be invaluable for many biochemical investigations. However, as was rather to be anticipated from earlier experiments (Flagg, 1941; McCallum & Hoshowsky, 1948), all our attempts (Fantes, Page, Parker & Smith, 1949) to label vitamin B_{12} by direct exchange with either radiocobalt chloride or hexammino radiocobaltic chloride were unsuccessful. We, therefore, attempted the biosynthesis of radioactive vitamin B_{12} by feeding physiological doses of radiocobalt chloride to two piglets over a 6-week period. Probably because the experiment was undertaken during the months of December and January, no significant amounts of the anti-pernicious-anaemia factor could be detected in the liver (cf. Dedichen, 1949). The distribution of radioactive cobalt in the pig carcasses was studied at the same time, and our results are described in this communication.

The earlier work on the metabolism of radioactive cobalt has been reviewed by Comar (1948). Copp & Greenberg (1941) administered radioactive cobalt to rats, and Greenberg, Copp & Cuthbertson (1943) extended the work to show the appearance of labelled cobalt in the bile, urine, faeces and liver of bile-fistula rats. Sheline, Chaikoff & Montgomery (1946) followed intravenously injected radioactive cobalt in the plasma, bile and pancreatic juice of dogs provided with bile and pancreatic fistulas. More recently, a group of workers at the Florida Agricultural Experiment Station (Comar, Davis & Taylor, 1946; Comar, Davis, Taylor, Huffman & Ely, 1946; Comar & Davis, 1947*a*, *b*) carried out experiments with physiological doses of cobalt on thirty-two cows, two pigs, twenty rabbits and 100 rats.

All the earlier work was performed with a mixture of ^{56}Co, ^{57}Co and ^{58}Co obtained by the deuteron bombardment of iron in the cyclotron. Recently, ^{60}Co, which is prepared by the neutron bombardment of ordinary cobalt in a nuclear pile, has become available for tracer work. A preliminary report by Lawrence (1947) on the metabolism of ^{60}Co, injected intravenously into mice, lists data for excretion rates and accumulation in various tissues; the data are in accord with the findings of the earlier workers, who used mixtures of ^{56}Co, ^{57}Co and ^{58}Co.

The American workers gave one dose of radioactive cobalt to each of their animals and then measured the rate of excretion and distribution in the body. In our experi-

ments, the radioactive cobalt was mixed with the food in physiological amounts so that there was a steady intake of the isotope over the whole period of the experiment.

EXPERIMENTAL

Two female pigs used in this investigation were about 10 weeks old when they were isolated in a special wooden sty. The experiment lasted 43 days, each pig receiving daily about 1·1 kg., rising to 1·8 kg., of diet, a total of 61 kg. being consumed during the experiment. The diet consisted of 50% fine wheat offals, 20% barley meal, 20% maize meal and 10% fish meal and contained 0·208 mg. ordinary cobalt/kg., to which had been added 0·038 mg. radiocobalt chloride/kg. (i.e. 13 μc. of ^{60}Co/kg.). Every μg. of ordinary cobalt was thus labelled with 0·053 μc. of ^{60}Co.

The various organs, after being dissected from the carcasses, were separately minced and a portion of each was weighed, dried to constant weight and digested in a mixture of nitric and sulphuric acids containing 0·1 ml. of 1·0% ordinary cobalt chloride as carrier. The residual solutions were diluted with 0·1 N-hydrochloric acid and a 10 ml. portion of each was transferred to the outer jacket of a liquid Geiger-Müller counter (cf. Veall, 1948). The counter was coupled to a Dynatron scaling unit (Dynatron Radio Ltd., Maidenhead) and the number of disintegrations/min. was recorded. The teeth and bone samples gave a sludge, which was centrifuged off before counting.

RESULTS

Our values for the distribution of radioactive cobalt in the pig are summarized in Table 1. The data for the weight of cobalt/100 g. wet organ were calculated on the assumption that every μg. of ordinary cobalt was associated with 0·053 μc. of ^{60}Co.

Table 1. *Distribution of radioactive cobalt in the pig*

Organ	Wt. of wet organ (g.)	Dry solids (%)	^{60}Co (μc./100 g. wet organ)	Cobalt (μg./100 g. wet organ)
	Pig no. 1			
Pituitary (entire gland)	0·19	35·0	<0·003	<0·05
Thymus	10·2	30·6	0·022	0·41
Adrenals	3·18	61·0	0·053	1·02
Ovaries	2·65	35·3	0	0
Uterus and Fallopian tubes	38·1	18·8	0·016	0·31
Brain	42·3	21·5	0·0053	0·10
Eyes	9·5	12·2	0·019	0·36
Heart	33·8	19·6	0·0101	0·19
Plasma	20·3	10·0	0·019	0·35
Serum	21·0	10·2	0·017	0·31
Red blood corpuscles (with citrate)	21·8	35·5	0·0069	0·13
Red blood corpuscles (without citrate)	20·3	34·9	0·0036	0·067
Lungs	37·5	19·4	0·017	0·32
Kidneys	30·7	18·4	0·101	1·9
Bladder	35·9	27·6	0·040	0·75
Tongue	23·7	25·5	0·0075	0·14
Salivary glands	24·6	22·2	0·012	0·22
Cardiac end of stomach	34·1	19·8	0·013	0·24
Pyloric stomach musculature	36·6	20·9	0·008	0·15
Pyloric mucosa	44·4	16·8	0·015	0·29

Table 1 (*continued*)

Organ	Wt. of wet organ (g.)	Dry solids (%)	^{60}Co (μc./100 g. wet organ)	Cobalt (μg./100 g. wet organ)
	Pig. no. 1 (*cont.*)			
Duodenum	35·1	14·7	0·015	0·29
Jejunum	33·7	15·3	0·023	0·43
Ileum	46·5	13·9	0·013	0·24
Caudal end of small intestine	36·8	13·5	0·021	0·39
Large intestine	30·5	25·8	0·020	0·37
Pancreas	48·1	24·0	0·0067	0·13
Spleen	21·1	19·5	0·022	0·42
Liver	39·0	29·6	0·051	0·95
Bile	15·0	—	0·0064	0·12
Gastrocnemius muscle	21·4	29·0	0·0091	0·17
Cartilage	15·7	30·7	0·046	0·87
Spinal cord	10·5	31·4	0·0075	0·14
Long bone	20·2	65·5	0·022	0·41
Bone marrow	8·0	76·2	0·018	0·33
Teeth	3·6	90·0	0·024	0·45
Hooves	22·0	52·3	0·020	0·37
Skin and hair	21·4	53·1	0·0106	0·20
	Pig no. 2			
Pituitary (entire gland)	—	—	—	—
Thymus	—	—	—	—
Adrenals	—	—	—	—
Ovaries	—	—	—	—
Uterus and Fallopian tubes	—	—	—	—
Brain	—	—	—	—
Eyes	—	—	—	—
Heart	30·1	19·2	0·018	0·33
Plasma	27·3	9·1	0·0096	0·18
Serum	—	—	—	—
Red blood corpuscles (with citrate)	25·5	33·5	0·0064	0·12
Red blood corpuscles (without citrate)	—	—	—	—
Lungs	31·1	18·0	0·024	0·46
Kidneys	35·2	19·2	0·106	2·0
Bladder	—	—	—	—
Tongue	—	—	—	—
Salivary glands	—	—	—	—
Cardiac end of stomach	24·5	25·0	0·027	0·51
Pyloric stomach musculature	33·5	19·0	0·016	0·30
Pyloric mucosa	44·5	16·4	0·017	0·33
Duodenum	27·1	17·6	0·023	0·44
Jejunum	29·5	12·2	0·015	0·29
Ileum	46·1	9·5	0·017	0·32
Caudal end of small intestine	—	—	—	—
Large intestine	39·5	22·8	0·018	0·33
Pancreas	—	—	—	—
Spleen	—	—	—	—
Liver	42·6	27·5	0·050	0·94
Bile	—	—	—	—
Gastrocnemius muscle	—	—	—	—
Cartilage	—	—	—	—
Spinal cord	—	—	—	—
Long bone	—	—	—	—
Bone marrow	—	—	—	—
Teeth	—	—	—	—
Hooves	—	—	—	—
Skin and hair	—	—	—	—

The relative amounts of radioactive cobalt found in the various organs or tissues (with the important exceptions of the pancreas, liver, bile, cartilage and bone) are in good agreement with the relative amounts found by Comar & Davis (1947*b*) in a pig 4 days after it had received an oral dose of 60 μg. of radioactive cobalt; the total amount found in our pigs was, of course, much greater. The relative amounts in the pancreas, liver and bile are about two-thirds lower, and those in the cartilage and long bone considerably higher, than the values reported by Comar & Davis.

Dr S. Ball determined the total cobalt in the liver from each pig by the β-nitroso-α-naphthol method (cf. Yoe & Barton, 1940) and obtained values of 2·3 and 3·2 μg./100 g. respectively. These amounts are much less than those found in ox liver, but are higher than the figures (0·95 and 0·94 μg./100 g.) calculated from the radioactivity results on the assumption that there had been a complete turnover of cobalt during the experiment. It must be inferred that less than half of the cobalt originally present in the liver had been replaced during the 43 days of the experiment.

SUMMARY

1. Physiological doses of radioactive cobalt were fed to two pigs for 6 weeks and the final distribution of radioactive cobalt in the carcasses was determined.

2. The relative amounts of radioactive cobalt found in most organs or tissues were similar to those found by Comar & Davis (1947*b*) in a pig after a single oral dose of radioactive cobalt.

3. The values for pancreas, liver and bile were lower, and those for cartilage and long bone higher, than those recorded by Comar & Davis.

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