Summary. The action of potassium chloride flooding on the cardiac, renal and adrenal effects of DCA-overdosage was investigated. The data indicate that there is a mutual antagonism between the actions of DCA and KCl on the heart and the adrenal. The role of the kidney in this antagonism is not known.

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Oral Administration of Vitamin B_{12} Containing Cobalt^{60} to Rats. (18501)

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Recent reports by Chow and associates (1,3) have demonstrated that when vit. B_{12} is administered orally to rats or humans, no microbiologic activity appears in the urine. They concluded from these observations that oral administration results either in the conjugation of vit. B_{12} to a form unavailable for the microorganism, in poor absorption in the intestinal tract, or in conversion to a form incapable of being excreted by the kidneys. Later they observed (unpublished work) that stool samples from rats receiving vit. B_{12} by mouth contained considerably more of this vitamin than stools excreted before administration. Although these data might be taken to indicate poor utilization of vit. B_{12}, this interpretation is complicated by the presence of some vit. B_{12} in the feces of untreated, normal rats and by the unreliability of the microbiologic assay for this vitamin in stool.

The availability of radioactive vit. B_{12} containing cobalt^{60} reported by Chaiet, Rosenblum and Woodbury(2) simplifies the analytical problem and permits a differentiation between the administered vitamin from that originally present, granting that the radioactivity due to cobalt^{60} found in the animal represents administered vit. B_{12}. It is therefore of interest to ascertain the distribution of vit. B_{12} as manifested by the presence of radioactive cobalt^{60} in urine and stool, as well as in certain organs of rats following oral administration. The results of such a study are reported below.

Experimental. Four normal rats weighing between 250 and 300 g were put into metabolism cages and fed a soy bean diet(3). Collections of urine and stool were made for 2 consecutive days. On the third day, 0.89 mg of radioactive B_{12},* with a specific activity of $\simeq 0.2$ microcurie per milligram, was administered orally to each rat. Daily collections of urine and fecal matter were again made for 3 to 4 consecutive days. On the fifth day after administration, the animals were sacrificed and the kidneys, liver, spleen, brain, testes and hearts removed. Each of these organs was homogenized *in toto* either with a Waring Blendor or with an Elvehjem homogenizer, and the suspensions were made up to a measured volume. An aliquot of each tissue homogenate was evaporated to dryness, wet ashed with H_2SO_4 after addition of several milligrams of ordinary cobalt to act

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* The radioactive vitamin employed in these experiments was provided by Merck & Co., Inc., on allocation by the Isotopes Division, U. S. Atomic Energy Commission.

as carrier, diluted to 10 ml in volumetric flasks, and assayed for cobalt^{60} content. One ml samples of blood were wet ashed directly. The stools collected before administration of vit. B_{12} were combined in a single sample, and stools collected after administration combined into a second sample. Aliquots of each were assayed after wet oxidation. The daily samples of urine were assayed directly, after appropriate dilution, for both radioactivity and microbiological activity. Radioactivity measurements were performed with a windowless counter (Q-Gas Counter) using evaporation residues in shallow aluminum planchets 3 cm in diameter. Under these conditions, the specific activity of the radioactive vit. B_{12} was 221,000 counts per minute (cpm) per mg; hence 197,000 cpm was administered per rat. The radioactivity of all samples was measured first using residues obtained by direct evaporation of the acid digests after neutralization with ammonia. Self-absorption corrections, based on ammonium sulfate residues, were applied to all samples as required. In the case of blood, liver, testes, heart, spleen and brain digests, the activities determined by direct evaporation of aliquots were quite low and not distinctly different from the controls. Accordingly, larger volumes of these acid digests were neutralized and treated with ammonium thiocyanate to form the blue cobalt thiocyanate complex, which was then extracted with a mixture of ethyl ether and isoamyl alcohol in a 4 to 1 ratio(4). The entire organic extract was evaporated in aluminum planchets for radiometric observation. Planchets were heated on a hot plate to remove small amounts of thiocyanate transferred to the planchets by the organic solvent. Kidney digests from 3 of the rats were also treated by this extraction method, as was one of the feces samples. Satisfactory agreement between both methods was obtained in these cases. A reagent control was run simultaneously with the test experiments. Basal urine and stool collections served as controls for excrement radioactivity. Two rats were included as controls for the blood samples.

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TABLE I. Distribution of Radioactivity After Oral Administration of Vit. B_{12} Containing Cobalt^{60}.

	Avg* activity, cpm	Stand. dev., ± cpm	Vit. B_{12} equivalent, μg
Liver	940	760	4.3
Kidney	920	270	4.2
Blood (1 ml)†	13	7	$\sim<0.06$
Spleen	13	20	$\sim<0.1$
Brain	19	15	$\sim<0.1$
Testes	17	20	$\sim<0.1$
Heart	—8	14	—

* In all cases, measured control activities were ±3 cpm. After correction for sampling schedule, etc., the calculated control values employed were 40 ± 18 cpm for liver, 4 ± 6 cpm for blood, and 30 ± 13 cpm for remaining organs.

† Avg of 3 rats.

Results. Results of the distribution measurements are reported in Table I which gives the average total cpm for the 4 rats, (corrected for the control radioactivity), the standard deviation for these net activities, and the quantities (in micrograms) of vit. B_{12} corresponding to these net activities. In Table II is reported the elimination of B_{12} (in micrograms) in the stool and urine. Results of microbiological assays of urine specimens, determined by means of *L. leichmannii* activity(5), are also included.

In addition to observing the radioactivity, the 24-hour urine samples were examined further to ascertain whether the radioactivity was truly present as vit. B_{12} rather than as free cobalt. The vitamin is known to be extractable by water-immiscible alcohols from concentrated aqueous solutions of ammonium sulfate(6). Subjecting the urine samples to this extraction procedure resulted in the removal of 80-100% of all activity. Furthermore the distribution of the extracted radioactivity between benzyl alcohol and water was close to that expected(2,7) for vit. B_{12}. These tests rule out the presence of radioactive in-

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TABLE II. Elimination (in μg) of Vit. B_{12} in Urine and Stool.

Sample	Time from admin.	Rat 1		Rat 2		Rat 3		Rat 4	
		Radio-act.*	Micro-bial	Radio-act.*	Micro-bial	Radio-act.*	Micro-bial	Radio-act.*	Micro-bial
Urine	—48	—	—	—	—	—		—	
	—24	—		—		—		—	
	+24	18.3	37.0	7.1	8.0	42.8	63.0	20.6	20.0
	+48	1.4	1.2	0.4	0.9	1.2	1.4	0.7	0.6
	+72	4.9	0.7	1.5	1.3	1.1	2.5	0	0.2
	+96	1.4	0.2	3.8	2.8	—	—		—
	Total	26.0	39.1	12.8	13.0	45.1	66.9	21.3	20.8
Feces	—48 (combined)	0	—	1.4†	—	13.0†	—	0	—
	+72 (combined)	415	—	582	—	67.4	—	192	—

* Basal urine activities were negligible. Stand. dev. of $\pm$0.4-0.6 μg per measurement were estimated from counting statistics.

† These basal feces activities were traced to contaminated glassware used for wet ashing.

organic cobalt, although the possibility that other vit. B_{12}-like compounds(6-9) are partly responsible for the radioactivity of the urine cannot be excluded.

Discussion. It is immediately evident from these results that the amount of orally administered vit. B_{12} excreted in the urine of rats is indeed small compared to the large dose administered. The largest percentage urinary excretion observed in these experiments amounted to about 5% of the initial B_{12} in the case of rat 3 over a three-day period. It is further apparent that the most marked radioactive B_{12} elimination in the urine occurs within the first 24 hours, and beyond this initial period the amount tends to diminish to very low values. Results of microbial assay parallel the radioactivity findings although, due to the presence of vit. B_{12} or other active components of the rat diet, they may indicate larger amounts of B_{12}. One must conclude from the cobalt^{60} content of the urine that only a fraction of the excessive dose of vit. B_{12} administered in these experiments is eliminated through the urinary tract, and that earlier observations to this effect are not due to the existence of the vitamin in conjugated form unavailable to the test microorganism.

Concomitant with the low vit. B_{12} content of the urine is the high cobalt^{60} content of the feces. In the case of rat 2, the radioactivity was equivalent to $\simeq$ 65% of the total radioactive B_{12} added to the diet. The fecal radioactivity of this and the other rats would undoubtedly have been even higher had the intestinal contents of the animals been removed for analysis at the time of autopsy. Whether this expedient would have accounted for the radioactive vitamin in its entirety is uncertain since a complete examination of the carcasses were not performed.

The dose of 0.89 mg of vit. B_{12} is, of course, far in excess of physiological requirements. Therefore, despite the low values found for B_{12} in the urine relative to the administered quantity, its very presence(4) proves that intestinal absorption occurs upon oral administration. This conclusion is supported by the presence of significant radioactivity, equivalent to $\simeq$ 0.5% of radioactive B_{12} administered in the kidneys and livers. The radioactivity of the blood, though small, is an indication of the passage of B_{12} through the blood stream. The quantities listed (Table I) for the remaining organs are doubtful, and probably represent maximal amounts. Additional work with high specific activity vitamin(10) is planned to ascertain the extent of transfer of the vitamin to these latter organs.

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