responding areas subtended by the various spectral curves throughout specific wavelength intervals. The 1100 to 1000 cm<sup>-1</sup> region proved to be the most informative one (see Fig. 2), for the intensity of the absorption band observed at 1065  $\rm cm^{-1}$  was always found to be higher for preparations showing enterotoxigenic activity than it was for preparations that were biologically inactive. In the latter, the magnitude of the 1065 cm<sup>-1</sup> absorption approached always closely that shown by the control media (Table 1).

Boiling of culture filtrates is known to cause the destruction of practically all hemolysins present (2) and is accompanied by the formation of a dense precipitate. The latter was removed from the specimens by filtration. Enterotoxin is not destroyed by the boiling process, however, and it represents the only biologically detectable active principle in the specimens examined.

Spectral differences due to the presence or absence of specific lysins remain to be adequately explored. The method reported appears, however, to allow for the detection of enterotoxin in appropriately treated preparations. It is planned to offer further details for publication elsewhere.

## LEO LEVI B. H. MATHESON F. S. THATCHER

Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Ontario

## References

- 1. F. S. Thatcher, B. H. Matheson, W. R. Simon,
- Can. J. Microbiol. 1, 401 (1955). 2. F. S. Thatcher and B. H. Matheson, Can. J.
- Microbiol. 1, 382 (1955).

27 June 1955

## **Reabsorption of Cobalt-60** from Urine and Bile Samples of Experimental Dogs

Investigators have reported on the metabolic fate of cobalt (1). They generally agree that cobalt is excreted mainly in the urine after intravenous injection and that a smaller fraction may be recovered from the bile. When cobalt is administered orally, a large fraction is excreted in the feces and considerable amounts are found in the urine. Our own work has confirmed these observations in chickens (2) and dogs (3). It is the aim of these studies to report the intestinal absorbability of the cobalt that is excreted in the urine and bile (4).

The hepatic bile and urine samples from an experimental dog (3) were employed. Pooled samples were collected between 0 and 4, 4 and 8, and 8 and 12 Table 1. Cobalt-60 recovery (percentage of injected dose) from intestinal tract of young chicks and absorption half-times when it was injected as urine or bile.

Injection	Amt. Co <sup>60</sup> injected	Intestinal Co <sup>60</sup> recovery	Half-time disappearance from intestine
	(µc)*	(%)†	(min)†
Co <sup>60</sup> SO <sub>4</sub>	0.406	$70.8 \pm 4.3$	$62.5 \pm 13.3$
0 to 4 hr Urine	0.303	$38.6 \pm 2.3$	$21.9 \pm 1.4$
4 to 8	0.298	$37.1 \pm 4.6$	$21.2 \pm 2.5$
8 to 12	0.223	$40.9 \pm 4.3$	$23.5 \pm 2.6$
Mean		38.9 ± 4.1	$22.2 \pm 4.2$
0 to 4 hr Bile	0.175	$41.1 \pm 3.6$	$23.5 \pm 2.2$
4 to 8	0.266	$45.1 \pm 3.5$	$26.3 \pm 2.4$
*** 8 to 12	0.309	$45.5 \pm 2.9$	$26.6 \pm 2.2$
Mean		$43.9\pm3.9$	25.5 ± 4.8

\* The specific activity was 0.5  $\mu$ c/ $\mu$ g. † Mean ± standard error.

hours after the initial intravenous injection of 10  $\mu$ c of Co<sup>60</sup> per kilogram (5). These were then diluted with physiological saline and were injected directly into the lumen of the gizzard of groups of 3-day old White Leghorn chicks as indicated in Table 1. Each group consisted of 11 birds. All chicks were killed  $\frac{1}{2}$  hour later. The intestinal tracts from above the proventriculus down to the cloaca were removed and ashed in a muffle furnace at 500 to 600°C for 5 hours. They were then weighed and counted under a thin mica end-window Geiger-Müller tube. Standards were prepared by adding known quantities of Co60 solution to the intestinal tract that had been removed from noninjected chicks and ashed and counted in the same manner. Since the weights of all the ashed samples were about the same (group means, 9.6 to 10.5 mg/cm<sup>2</sup>), no self-absorption correction was applied.

The data are presented in Table 1. When inorganic Co<sup>60</sup> was injected, the intestinal recovery of Co60 was significantly greater than that of Co<sup>60</sup> injected as urine or as bile. It will be noted, accordingly, that the half-time of disappearance of Co<sup>60</sup> from the intestine was longer in the group that received inorganic Co<sup>60</sup> than it was in those that received urine or bile samples. It has been found that the turnover rate of Co<sup>60</sup> in dogs is faster for its amino acid complex forms than for its inorganic form (6). It might be possible that inorganic Co60 administered to a dog is complexed before it is excreted in the urine or the bile.

Paper partition chromatography of the urine and bile samples, using autoradiograms to locate the spots containing Co<sup>60</sup>, was also studied. Although inorganic Co<sup>60</sup> apparently accounted for a large majority of the radioactivity, additional radioactive components were present in both the urine and bile samples. However, in no specific case was it possible to conclude that more than a very minute trace of the Co<sup>60</sup> in either urine or bile samples was in the form of vitamin  $B_{12}$ (7). The existence of some forms of Co<sup>60</sup> other than its inorganic form in the intestinal wall and its contents of the chicken (2), in the blood plasma of the dog (8), and in the tissues of the sheep (9) has been discussed.

In summary, a form (or forms) of Co<sup>60</sup> other than its inorganic form was found in the bile and in the urine samples that were collected from experimental dogs. The Co<sup>60</sup> in these samples is reabsorbed from the gut of young chicks at a considerably faster rate than inorganic Co<sup>60</sup> is absorbed.

> CHENG-CHUN LEE\* L. F. WOLTERINK

Department of Physiology and Pharmacology, Michigan State University, and Michigan Agricultural Experiment Station, East Lansing

## **References and Notes**

- C. L. Comar and G. K. Davis, J. Biol. Chem. 170, 379 (1947);
  C. L. Comar, G. K. Davis, R. F. Taylor, Arch. Biochem. 9, 149 (1946);
  C. L. Comar et al., J. Nutrition 32, 61 (1946);
  D. M. Greenberg, D. H. Copp, E. M. Cuthbertson, J. Biol. Chem. 147, 749 (1943);
  G. E. Sheline, I. L. Chaikoff, M. L. Montgomery, Am. J. Physiol. 145, 285 (1945-1946).
  C. C. Lee and L. F. Wolternik, Poultry Sci. 34 764 (1955).
- 2. 34, 764 (1955). ——, Am. J. Physiol. 183, 167 (1955).
- This report is published with the approval of the director of the Michigan Agricultural Experiment Station as journal article No. 1772. Our work was supported in part by the Division of Biology and Medicine of the U.S. Atomic
- Energy Commission. Cobalt-60, as  $Co^{00}SO_4$ , was obtained from Trac-erlab, Boston, Mass. The stock solution contain-ing 400 µg of cobalt per milliliter of 0.1N HCI solution was diluted to about 70  $\mu$ g/ml (*p*H 2) with physiological saline. The specific activity
- of the Co<sup>60</sup> was 0.5 µc/µg. N. I. Berlin and W. Siri, *Am. J. Physiol.* 164, 221 (1951); C. C. Lee, Ph.D. thesis, Michigan 6. State Univ
- State Univ. Cobalt-60-labeled vitamin  $\mathbf{B}_{12}$  was spotted on the paper chromatogram for identification. It was kindly supplied by C. Rosenblum of Merck and Company, Inc., Rahway, N.J. C. C. Lee and L. F. Wolterink, Am. J. Physiol. 102, 102, 1055
- 8. 183, 173 (1955).
- R. A. Monroe et al., Proc. Soc. Exptl. Biol. Med. 80, 250 (1952).
- Present address: Pharmacology Division, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis 6, Ind.

18 August 1955