Laboratories, Standard Brands, Inc., Stamford, Conn.
8. E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry* (Thomas, Springfield, Ill., 1948), p. 122.
9. M is the symbol for the midpiece C′ fraction. It contains C′1, C′3, and a little C′4.
10. E is the symbol for the endpiece C′ fraction. It contains C′2, some C′3, and much C′4.
11. G. C. Brown, *J. Immunol.* **46**, 319 (1943).

12 August 1955

Detection of Staphylococcus Enterotoxin by Infrared Spectrophotometry

This communication describes briefly the results of an infrared spectrophotometric examination of boiled and lyophilized preparations that were obtained from cultures of enterotoxigenic and nonenterotoxigenic staphylococci (*Micrococcus pyogenes* var. *aureus*) in accordance with the "cold-ethanol" method developed by Thatcher and Matheson (*1*). The products were known to contain variable amounts of α-, β- and δ-hemolysins and differed widely in enterotoxigenic activity.

Exploratory experiments were carried out on specimens mounted between two silver chloride disks in a specially constructed demountable microcell. A drop of the aqueous concentrate was placed on one of the disks, a stream of dry nitrogen was passed over it to remove excess solvent, and the specimen was thoroughly dried over phosphorus pentoxide. Then its absorption was measured in a Perkin-Elmer double-beam recording infrared spectrophotometer. Remarkable contour similarity was noted to exist among the various spectral curves that were obtained by this procedure, and it soon became apparent that only a quantitative evaluation of the absorption characteristics of the different preparations might prove to be of diagnostic value.

Accordingly, the specimens were finely powdered in a mechanical grinder until they would pass through a 250-mesh sieve (U.S. Standard Sieve Series No. 230). An accurately weighed portion of 5 mg was then mixed intimately with 995 mg of ACS reagent grade potassium bromide that had been prepared similarly and dried overnight at 125°C. A 200-mg aliquot of the mixture was subjected in a vacuum for about 5 minutes to a pressure of 10,000 lb/in.^{2} The absorbancy of the clear disk thus produced was measured over the frequency range extending from 4000 to 650 cm^{-1}. A potassium bromide disk prepared under comparable conditions was placed in the path of the reference beam to compensate for absorption by the reagent. After each experiment, the disks were weighed accurately in order to determine the amount of specimen per sample. The variations between different disks never exceeded 1 percent.

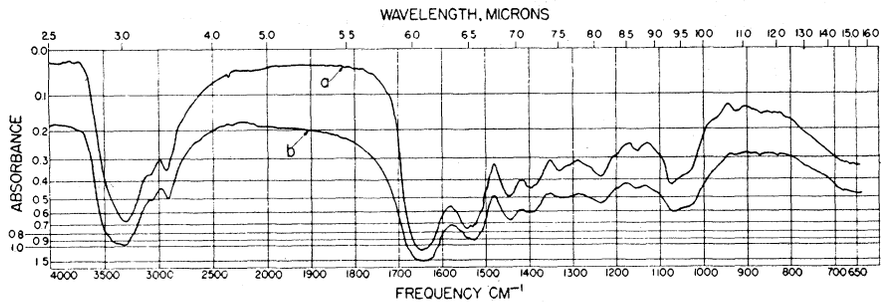

Fig. 1. Infrared absorption curves of 12069α Dolman preparation measured by the silver chloride technique (*a*), and the potassium bromide technique (*b*).

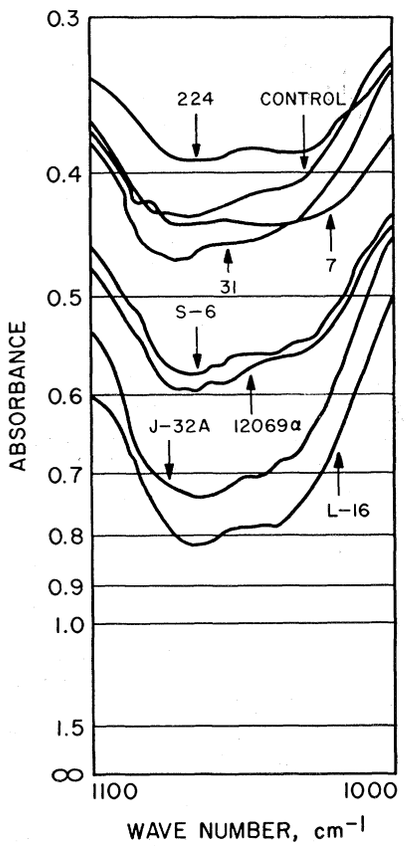

Fig. 2. Infrared absorption of seven strains of *M. pyogenes* var. *aureus* throughout the 1100–1000 cm^{-1} region.

Table 1. Correlation between enterotoxicity and infrared absorption of a specific fraction* of the filtrates of seven strains of *M. pyogenes* var. aureus.

Strain	Enterotoxicity (cat test)			Area under curve from 1100 to 1000 cm^{-1} (cm^2)
	Material injected† (mg)	Cats injected (No.)	Cats vomiting in 2 hr (No.)	
L16	2	4	4	15.8
J-32A	2	4	3	18.5
S6	2	4	3	23.7
	5	1	1	
12069α	2	4	4	23.8
Control media	2–5	4	0	32.4
31	2–5	4	0	30.4
7	2	4	0	30.7
224‡	2	4	0	33.8
	5	1	0	

* Water-soluble precipitate obtained by the "cold-ethanol" procedure of Thatcher, Matheson, and Simon (*1*).
† All material dissolved in 2 ml of 0.95-percent saline prior to injection.
‡ This strain is usually weakly enterotoxic but was found to contain no enterotoxin in this particular preparation.

Figure 1 shows two spectral curves obtained on one of the preparations (12069α Dolman) using both the silver chloride and potassium bromide techniques. Strong N—H and characteristic C—H stretching vibrations are observed at 3400 cm^{-1} and 2900 cm^{-1}, respectively. The marked absorptions noted at 1650 and 1540 cm^{-1} are indicative of the presence of polypeptide bonds, while the characteristic band occurring at 1065 cm^{-1} may be considered to be associated with ester linkages such as —C—O or C—O—P that are found in phospholipids.

Because all samples were treated identically by the pressed potassium bromide technique, their absorbancies could be compared by accurately measuring cor-

responding areas subtended by the various spectral curves throughout specific wavelength intervals. The 1100 to 1000 cm^{-1} region proved to be the most informative one (see Fig. 2), for the intensity of the absorption band observed at 1065 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^{-1} 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.

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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 hours after the initial intravenous injection of 10 μc of Co^{60} 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 ½ 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 Co^{60} 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^2), no self-absorption correction was applied.

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^{60} injected	Intestinal Co^{60} recovery	Half-time disappearance from intestine
	(μc)*	(%)†	(min)†
$Co^{60}SO_4$	0.406	70.8 ± 4.3	62.5 ± 13.3
0 to 4 hr Urine	0.303	38.6 ± 2.3	21.9 ± 1.4
4 to 8	0.298	37.1 ± 4.6	21.2 ± 2.5
8 to 12	0.223	40.9 ± 4.3	23.5 ± 2.6
Mean		38.9 ± 4.1	22.2 ± 4.2
0 to 4 hr Bile	0.175	41.1 ± 3.6	23.5 ± 2.2
4 to 8	0.266	45.1 ± 3.5	26.3 ± 2.4
8 to 12	0.309	45.5 ± 2.9	26.6 ± 2.2
Mean		43.9 ± 3.9	25.5 ± 4.8

* The specific activity was 0.5 μc/μg. † Mean ± standard error.

The data are presented in Table 1. When inorganic Co^{60} was injected, the intestinal recovery of Co^{60} was significantly greater than that of Co^{60} injected as urine or as bile. It will be noted, accordingly, that the half-time of disappearance of Co^{60} from the intestine was longer in the group that received inorganic Co^{60} than it was in those that received urine or bile samples. It has been found that the turnover rate of Co^{60} in dogs is faster for its amino acid complex forms than for its inorganic form (*6*). It might be possible that inorganic Co^{60} 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^{60}, was also studied. Although inorganic Co^{60} 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^{60} in either urine or bile samples was in the form of vitamin B_{12} (*7*). The existence of some forms of Co^{60} 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^{60} other than its inorganic form was found in the bile and in the urine samples that were collected from experimental dogs. The Co^{60} in these samples is reabsorbed from the gut of young chicks at a considerably faster rate than inorganic Co^{60} is absorbed.

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3. ———, *Am. J. Physiol.* 183, 167 (1955).
4. 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.
5. Cobalt-60, as $Co^{60}SO_4$, was obtained from Tracerlab, Boston, Mass. The stock solution containing 400 μg of cobalt per milliliter of 0.1*N* HCl solution was diluted to about 70 μg/ml (*p*H 2) with physiological saline. The specific activity of the Co^{60} was 0.5 μc/μg.
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7. Cobalt-60-labeled vitamin 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.
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