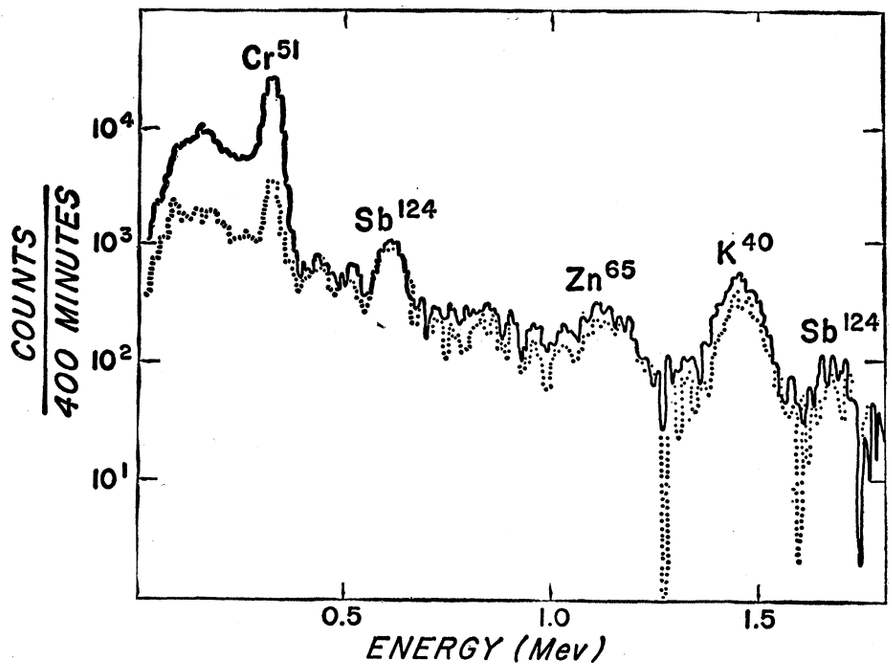

Fig. 1. Gamma-ray spectra of precipitates from 560-liter samples of sea water collected off Newport, Oregon, June 1965. Dotted line, Cr(III), Mn(II), and Zn(II) carrier were added; solid line, Sb(III), Cr(III), Mn(II), and Zn(II) were added. Enhancement of Cr^{51} is attributed to reduction of Cr(VI) to Cr(III) by Sb(III). The 1.69-Mev photopeak for Sb^{124} is not smooth because the counting rate is so low that random variations are seen.

was essentially complete, whether carrier was added or not. In the case of Cr(VI), recovery was incomplete and results were erratic when no carrier or Cr(III) carrier was used. Carrier Cr(VI) consistently reduced the yield. However, either reducing agent was effective in giving complete recovery.

Similar results were obtained at sea with proportionately larger samples and precipitates (Table 2). Laboratory and shipboard precipitates were made with 45 mg of Fe(III) and 0.64 ml of 28 percent NH_4OH per liter of solution. Similarly, carriers and reducing agents were added in the same proportion (5.3 mg of chloride salts or $K_2Cr_2O_7$ per liter) to the 250-ml laboratory tests and to the 560-liter shipboard samples. Aboard the U.S. Coast Guard cutter *Modoc*, in June, sampling was extended over an 18-hour period, during which time the ship drifted through waters of varying salinity. It was therefore necessary to rectify the counts to allow for the changing fraction of Columbia River water in our samples. All counts were corrected to a salinity of 29.2 per mil.

To obtain truly duplicate samples, the tests were repeated in August aboard the research vessel *Yaquina*. Levels of Cr^{51} were much lower because the tongue of low-salinity water observed in June (*8*) had spread greatly and become saltier. Rectification of these data was not required, since salinity values did not vary during the tests.

In every case maximum yield of Cr^{51} from sea water occurred when a reducing agent was added prior to precipitation. We conclude that Hanford-induced Cr^{51} remains principally in hexavalent form in the ocean. This conclusion is supported by the "hold-back" effect of Cr(VI) carrier. Since the Cr^{51} that we measure off Newport has been in contact with sea water for weeks or even months, we conclude that reduction of Cr(VI) to Cr(III) must occur only very slowly if at all. Any Cr(III) in the ocean or in the river would associate with particles and be lost to the sediments, while Cr(VI) would remain in solution. We have measured Cr^{51} in filtered sea water up to 525 km from the mouth of the Columbia River, and the results indicate that losses due to reduction to Cr(III) are small.

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9. Supported by contract AT(45-1)1750 with the AEC and by training grant 5T1-WP-59-02 from USPHS.

10 January 1966.

Chloramphenicol-Specific Antibody

Abstract. *Antibody to the antibiotic chloramphenicol was obtained by immunizing rabbits with a chloramphenicol derivative coupled to bovine gamma globulin. Production of antibody was demonstrated by the precipitin and complement fixation reactions with "reduced chloramphenicol" coupled to rabbit serum albumin as the test antigen. Specificity of the antibody was confirmed in that crystalline chloramphenicol completely inhibited complement fixation and precipitin reactions. "Reduced chloramphenicol" coupled to human serum albumin provides an antigen for the detection of antibody to chloramphenicol, if it occurs in human serum in dyscrasias. With quantitative complement fixation, as little as 10^{-5} μg (4×10^{-14} mole) of chloramphenicol was detectable by the inhibition assay.*

During the course of an investigation into the effect of chloramphenicol on animal cells growing in tissue culture, it became desirable to obtain antibody to the antibiotic. To determine whether this antibiotic would serve as an antigenic determinant, its nitro group was reduced (*1*) to the amine (Fig. 1) and coupled by diazotization (*2*) to the carrier protein, bovine gamma globulin (BGG). Detecting antigens were similarly prepared from the reduced chloramphenicol (RCAP) diazotized to rabbit serum albumin (RSA) and human serum albumin (HSA). Spectrophotometric analysis for azo groups (*3*) indicated an aver-

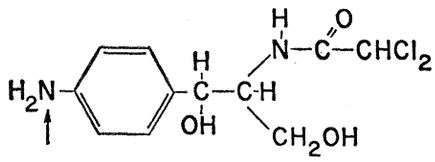

Fig. 1. "Reduced chloramphenicol" (RCAP): D-threo-1-*p*-aminophenyl-2-dichloroacetamido-1,3-propanediol.

age of five molecules of reduced antibiotic per molecule of carrier protein.

Six New Zealand rabbits were injected in the toe pads and subcutaneously with 0.5 mg of RCAP-BGG incorporated into Freund's complete adjuvant. Booster doses of 0.5 mg of antigen in saline were administered intravenously at 6- to 8-week intervals. Animals were bled 10 days after booster doses.

By using RCAP-RSA as the test antigen, the rabbit antiserums could be examined for chloramphenicol-specific antibody, since no cross-reaction between rabbit antiserum to RCAP-BGG and RSA was measurable.

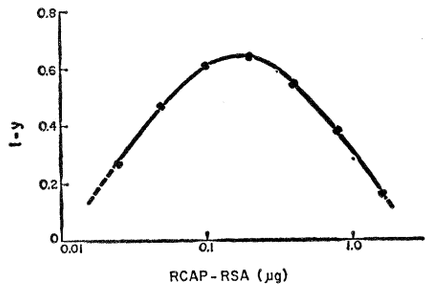

Fig. 2. Complement fixation curve obtained with antibody to RCAP-BGG diluted 1:1000 and tested against RCAP-RSA. The ordinate, 1—y, is proportional to the fraction of complement fixed; abscissa, increasing quantities of the antigen RCAP-RSA (μg). The equivalence point is approximately 0.2 μg of RCAP-RSA.

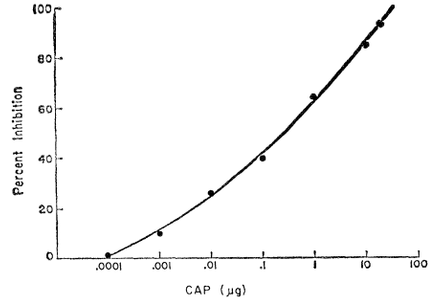

Fig. 3. Percentage inhibition by crystalline chloramphenicol (CAP) of the complement fixation by antibody to RCAP-BGG, 1 to 700 dilution, and 0.4 μg of RCAP-RSA.

After the first booster dose four of the six rabbits developed antibody as measured by the precipitin reaction (*4*) and by a quantitative micromethod for measuring complement fixation (*5*). After the third series of booster injections all rabbits had chloramphenicol-specific antibody averaging about 150 μg of antibody nitrogen per milliliter (*6*).

The complement fixation curve, obtained with antiserum to RCAP-BGG at a 1:1000 dilution and the antigen RCAP-RSA, is shown in Fig. 2. The equivalence point ($1 - y = 0.64$) is reached with 0.20 μg of RCAP-RSA; and 0.025 μg gives significant complement fixation ($1 - y = 0.27$). The 0.025 μg of RCAP-RSA contains approximately 0.0004 μg of chloramphenicol.

Confirmation of the specificity of the antibody to chloramphenicol was provided by the classical hapten inhibition test. The reaction mixture contained antibody to RCAP-BGG at 1:700, 0.4 μg of RCAP-RSA, 1.5 $C'H_{50}$ units (*5*), and increasing amounts (10^{-4} to 20 μg) of crystalline chloramphenicol (*7*). The addition of 20 μg of chloramphenicol virtually eliminated complement fixation (Fig. 3), and significant inhibition was detectable with 10^{-3} μg of the antibiotic. The sensitivity of the inhibition assay could be increased by appropriate changes in the amount of antiserum used in the assay, and as little as 10^{-5} μg of the antibiotic could be reproducibly detected by this method.

Utilizing both the complement fixation and the inhibition of complement fixation techniques in studies with HeLa cells grown in the presence of chloramphenicol (*8*) we have been able to detect approximately 10^{-5} μg of the antibiotic in about 10^6 HeLa cell nuclei, and between 0.01 and 0.5 mole per mole of ribosomes in *Escherichia coli* ribosomal preparations (*9*). Analysis of the supernatant for chloramphenicol after incubation with ribosomes confirmed its binding by *E. coli* ribosomes.

The demonstration of antibodies specifically directed to chloramphenicol and the sensitivity of the hapten inhibition test in complement fixation offer the possibility that reagents can be developed to examine some aspects of the toxicity of chloramphenicol in human beings. If this toxicity, manifested by destruction of the cells in the bone marrow, were to have an immune basis, human antibody to chloramphenicol present in the patient's serum (*10*) might be detectable with RCAP-HSA as antigen. On the other hand, the presence of chloramphenicol in particular cellular elements or tissues could be determined by the inhibition test with hyperimmune rabbit antiserum. The occurrence in the serum of antibody to chloramphenicol or the cellular localization of the drug in such patients would provide valuable information toward clarification of the etiology of this disease.

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References and Notes

1. The reduction of chloramphenicol catalyzed by platinum oxide, which we performed, was reported to result in side-reaction products by Mildred C. Rebstock, Parke, Davis and Company. Therefore, the experiments reported in this paper were performed with D-threo-*p*-aminophenyl-2-dichloro-acetamido-1, 3-propanediol ("reduced chloramphenicol"), batch 4633-4000$_4$, isolated as the crystalline hydrochloride after a selective dichloroacetylation of D-threo-1-*p*-aminophenyl-2-amino-1, 3-propanediol. This compound was supplied by Dr. Rebstock.
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6. Confirmed with help of Dr. W. O. Weigle, Scripps Clinic and Research Foundation, La Jolla, Calif.
7. Crystalline chloramphenicol, batch X7855, supplied by Parke, Davis, and Company.
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too anticomplementary for satisfactory analysis.

9. Five grams each of *E. coli* K 12 with and without chloramphenicol (500 mg/g) were broken in a French press, centrifuged (Spinco Model L) consecutively at 15,000, 30,000, and 105,000*g* for 10, 30, and 90 minutes. The final pellets (approximately 125 mg each) were standardized by dilution in buffer and analysis at 260 mμ in the Zeiss spectrophotometer.
10. Dr. J. Kumate, Hospital Infantil, and Dr. C. Biro, National Institute of Cardiology, Mexico, D.F., provided 84 serums currently under study from patients with hematologic disorders who had been exposed to chloramphenicol.
11. With the technical assistance of J. H. Douglass and editorial advice from Professor S. E. Mills. Supported by PHS grant AM 09207.

24 January 1966

Iodoinsulin Used To Determine Specific Activity of Iodine-131

Abstract. *Anodal mobility of iodoinsulin on starch-gel electrophoresis increases progressively as the number of iodine atoms substituted in the molecule increases. The iodine content of iodine-131 solutions is determined by comparison of autoradiographic patterns of insulin heavily labeled with iodine-131 and of insulins lightly labeled with iodine-131 and known quantities of iodine-127.*

A simple experimental determination of the specific activity of nearly carrier-free I^{131} has heretofore been lacking. Since I^{131} itself contains only about 8 μg of iodine per curie, direct chemical measurement of the iodine content of I^{131} solutions requires a prolonged wait for physical decay to lessen the radiation hazard involved in handling measurable amounts of iodine. The amounts of the isotopes I^{127} (stable), I^{129} (half-life, 1.6×10^7 years), and I^{131} (half-life, 8.05 days) produced by neutron irradiation of tellurium targets can be calculated theoretically from the isotopic abundance of tellurium isotopes and their cross sections for neutron capture. However, considerable uncertainty exists in the cross-section data, and small but significant and unknown amounts of I^{127} may be present originally in the tellurium targets or picked up as contaminant during processing. Even greater difficulties may confront the estimation, from fission yields, of the specific activity of I^{131} obtained as a fission product.

We now present a relatively simple method for determining the specific activities of I^{131} preparations. We have aimed at accuracy only within 30 percent, but greater accuracy should be possible with little additional effort. Our method is based on the fact that insulins labeled with small amounts of I^{131} in the presence of varying quantities of I^{127} exhibit series of discrete spots on starch-gel electrophoresis; the series appear to correspond to iodinated insulins having different ratios of iodine to protein (*1*). The chemical iodine content of a batch of I^{131} can thus be estimated by comparing the electrophoretic pattern of highly labeled I^{131}-insulin with patterns obtained with known amounts of I^{127}.

Freshly dissolved bovine crystalline insulin (Lilly lot No. 719106) was iodinated by the method of Hunter and Greenwood (*2*), with minor modifications (*3*). The contents of the reaction vessels were immediately transferred to tubes containing 40 to 300 μl of plasma so that the final I^{131} concentrations were approximately the same for all preparations. Forty microliters of each mixture were immediately placed in slits of a starch gel for electrophoresis (*4*), and a small sample was further diluted in plasma and applied to paper strips for analysis by hydrodynamic-flow chromatoelectrophoresis (*5*). In the latter procedure, undamaged I^{131}-insulin remains absorbed to the paper at the origin, damaged labeled fractions migrate with the plasma proteins, and iodide migrates further anodally.

After approximately 6 to 7 hours at 8 v/cm or 12 to 14 hours at 4 v/cm, starch gels were removed from the mold, wrapped in Parafilm, and placed in contact with x-ray film for autoradiography. Multiple films were obtained with exposures varying from 3 to 30 minutes; in this way the relative densities of the major spots could be evaluated from the short exposures, and the presence of less-active spots could be brought out in the longer exposures. Although different observers using naked-eye comparison were in good agreement on the iodination levels of highly labeled I^{131}-insulin, the intensities of spots on the autoradiographs were more objectively evaluated by transmission densitometry (*6*).

When starting ratios of I^{131} to insulin were about 1000 to 1800 mc of I^{131} per milligram of insulin, the efficiency of iodination was usually about 70 to 90 percent as determined by chromatoelectrophoresis on paper. Most of the radioactivity was distributed between the first and second "pre-albumin" spots

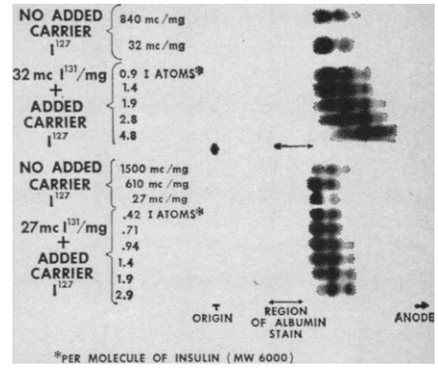

Fig. 1. Autoradiograph of starch-gel electrophoresis of insulin-I^{131} preparations. The numbers given for iodine atoms per molecule of insulin indicate the average value for the preparation; they are calculated as the product of the starting ratios and the percentage iodination. The molecular weight of the beef insulin monomer was taken as 6000 (approximation). Since the molecular weight is in fact 5734, the iodine numbers in this and succeeding figures are more precisely 4.5 percent higher than shown.

on starch-gel electrophoretograms, but a more-anodal third spot was always evident (Fig. 1); in more prolonged exposures a weak fourth spot was frequently brought out and a still weaker fifth spot was occasionally detectable (Fig. 2). When the same I^{131} was used at 15 to 35 mc of I^{131} per milligram of insulin, most of the insulin-bound radioactivity migrated in the first pre-albumin spot and only minute amounts were ever present beyond the second spot; occasionally, the efficiency of iodination was less than 70 percent in these preparations, possibly as a consequence of the very low iodine concentrations. When the same small quantity of I^{131} was supplemented with increasing quantities of I^{127} the iodination yields again increased (*7*) and an electrophoretic pattern showed an increasing number of spots, with a progressively more-anodal localization of radioactivity (Fig. 1, *8*).

The average ratio I^{127} : insulin was calculated as the product of the starting ratios (usually an integral or half-integral number of I^{127} atoms per molecule of insulin monomer—molecular weight, 6000) and the percentage of I^{131} bound to the insulin, as determined from the chromatoelectrophoretic analysis.

At 1 atom or less of I^{127} per molecule the intensity was always greatest in the first spot. At 1.5 atoms per molecule the second spot was slightly more