vascular compartments. During the period of our experiment radioactivity in the blood remained constant and uniform, as was evident from radioassay of blood samples withdrawn at 5 minute intervals and similar specific radioactivity found in blood drawn from the right or left eye.

The bioassay proved useful and reliable in our laboratory under a variety of testing conditions. While our experience has been exclusively with mice, the technique can be used with other small laboratory animals. It eliminates the need for such methods of detecting mosquito biting as looking for mosquitoes with distended abdomens, looking for the sign of a bite on shaved skin, or visual inspection of landing, procedures of obviously doubtful value.

Summary. A laboratory method for measuring the number of mosquitoes that had bitten and the amount of blood ingested during exposure is described. The procedure was successfully used in testing mosquito repellents administered to living mice.

1. Dethier, V. S., *Ann. Rev. Entomol.*, 1956, v6, 181.

2. Riley, V., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 751.

3. Bar-Zeev, M., Schmidt, C. H., *J. Econ. Entomol.*, 1959, v52, 268.

4. Boormal, J. P. T., *Ann. Trop. Med. and Parasit.*, 1960, v54, 8.

5. Lal, H., Ph.D. Thesis, Univ. of Chicago, 1962.

Received April 24, 1963. P.S.E.B.M., 1963, v113.

Separation and Quantitative Recovery of Iron and Cobalt from Biological Samples.* (28487)

PAUL VANDER KOOI, BEATRICE HEAGAN AND JAMES T. LOWMAN
(Introduced by W. Krivit)
Department of Pediatrics, University of Minnesota, Minneapolis

Trace metal analysis in biological samples has been accomplished with difficulty by conventional means. Recent reports of activation analysis demonstrate its extreme sensitivity and usefulness in this area(1). Neutron activation of biological samples produces an array of isotopes whose gamma ray spectra may obscure the element for which quantitation is desired. When the specimen contains large numbers of elements at extremes of concentration the various radio-spectral techniques for quantitation become either impractical or imprecise. The quantitation of Fe^{59} and Co^{60} in samples exemplifies these problems(2). The method outlined below is a relatively simple ion-exchange column separation for iron and cobalt. While developed specifically for separation of these 2 elements in activated specimens, its demonstrated excellent quantitative recovery would suggest broader applications for biological trace metal research.

Materials and methods. Pyrex columns 26 cm × 1 cm topped by an 8 cm funnel were used. These were plugged with glass wool and 15 ml of a slurry, made one part Dowex-1 (100 – 200 mesh) to one part distilled water, were added. The columns, which were filled to aproximately 18 cm were then charged utilizing concentrated HCl.

High specific activity ferrous citrate§ (Fe^{59}, 26.5 mc/mg) and cyanocobalamin§ (Co^{57}, 750 μc/mg B_{12}) were added to fresh pooled bovine serum. The final concentration of added ferrous citrate was approximately 0.02 μg/ml plasma and of B_{12} approximately 0.5 μg/ml plasma. One ml aliquots of this plasma were then pipetted into small aluminum foil containers which were heated in a drying oven at 100°C until the plasma became

* This work was supported in part by U.S.P.H.S. grant and the Graduate School, Univ. of Minnesota.

§ Abbott Laboratories, Isotope Division, Oak Ridge, Tenn.

a firm gel (20-30 min). This procedure is routinely used in this laboratory when preparing samples for activation analysis(3).

The aluminum foil encapsulated pellets were then placed in a 50 ml Erlenmeyer flask and one ml of concentrated HCl was added. The acid was allowed to react with the aluminum for approximately 15 minutes at which time 5 ml concentrated HCl was added and the flask left on a hot plate at 150°C until it evaporated to dryness.† The flasks were then cooled and 5 ml of concentrated HNO_3 were added and the mixture was again heated at 150°C to dryness, then at 300°C for 2 hours. This last heating seemed necessary to release the Co from the B_{12} molecule, allowing it to exchange with the resin. After cooling, 5 ml of concentrated HNO_3 were added and the flask heated to 150°C until approximately 2 ml remained. The mixture was allowed to cool and 10 ml of concentrated HCl were added and the flask again heated at 150°C until the brown nitric fumes were no longer evolved. Five ml of conc. HCl were added at which time more nitric fumes were evolved. The heating was continued until fumes were no longer seen, then 5 ml conc. HCl were added and the samples were immediately poured onto the columns.

Each flask was immediately rinsed with 4 ml concentrated HCl and this is added to the column. After the conc. HCl wash had passed into the resin 4 ml of 4N HCl were used to wash the flask and this added to the column. Ten ml of 4N HCl were then added directly to each column. The cobalt was eluted in this fraction. When the 4N HCl had completed its run the iron was eluted by adding 20 ml of 0.5N HCl to each column. Each of these fractions were collected in clean 50 ml beakers.

In order to calculate recovery, the following precautions were observed. The same pipette was used to measure each of the 1 ml aliquots of plasma. Six 1 ml aliquots of the original plasma were set aside to serve as standards. Each fraction, concentrated HCl, 4N HCl and 0.5N HCl was collected in a 50 ml beaker of uniform size. Each fraction was diluted to the same level in order to assure consistent counting geometry. Co^{57} was chosen as the tracer since its γ-ray spectrum allows its quantitation when mixed with Fe^{59}. The pre-run standards and each fraction were then analyzed on a 400-channel multi-channel analyzer. The integrated counts under the 1.1 and 1.28 mev. peaks of Fe^{59} and the 0.12 mev. peak of Co^{57} were computed and corrected for background. Since Fe^{59} will contribute counts in the low peak area of Co^{57} it was necessary to use a pure Fe^{59} standard to calculate the ratio of counts in the photo peak to the area covered by the Co^{57} peak. This ratio remains constant, thus in mixed samples the counts in the Co^{57} peak contributed by Fe^{59} can be easily calculated. Co^{57} has no activity higher than its 0.12 mev. peak, thus it will not contribute counts in the area of the Fe^{59} peaks.

† A few drops of caprylic alcohol will decrease the foaming associated with the release of protein from the foil containers.

TABLE I.

No. samples	Comment	Mean % ± S.D. Fe^{59} recovered	Mean % ± S.D. Co^{57} recovered
54	With carrier	99.21 ± 1.84	101.20 ± 2.51
18	Without "	99.00 ± .92	98.87 ± 2.21
Total 72		99.16 ± 1.51	100.62 ± 2.52

The mean recovery of tracer Fe and Co from 72 samples in which both elements were present and which were separated quantitatively by method described in text.

Results. The counts recovered were converted to percent of the pre-run sample activity. Table I shows mean recoveries and standardization for 72 samples which contained both Fe^{59} and Co^{57}. More easily to define separation by visual means in the early studies, carrier was added to each sample. This increased concentration gave easily discernible color bands on the columns. However, as listed in Table I, the last 18 samples were separated and analyzed without carrier. The results of these 18 samples are in close agreement with the samples in which carrier was used. These results demonstrate separation and recovery of the elements at true trace concentrations.

Discussion. The separation of metals in the Ni, Mn, Co, Cu, Fe, and Zn group using the quaternary amine polystyrene divinyl ben-

zene resin, Dowex-1 was reported by Kraus and Moore(4). Quantitative separation of salts of these elements in pure solutions is relatively simple. However, the numerous elements and proteins of biological specimens required considerable hydrolysis in concentrated acids to prepare them for quantitative separation.

This procedure is very useful for specimens which have undergone neutron activation. The aluminum foil containers can be digested with the sample. This eliminates the error introduced when samples must be emptied from their containers. If the sample can be introduced directly to the flask the first concentrate HCl additions are unnecessary. The entire procedure requires approximately 16 hours to complete. It requires minimal attention and can be interrupted at any stage except following the last addition of HCl, when it must be immediately poured onto the column.

The separation of iron and cobalt from biological tissues has application in isotope studies when recovery of either or both elements is required. While the recovery reported here was accomplished from bovine plasma, the procedure is capable of this separation from high protein tissue such as packed red cells. Theoretically the technique will allow quantitative recovery of iron and cobalt in trace concentrations from any specimen that can be digested by concentrated acid.

Summary. Trace amounts of iron and cobalt are separated and quantitatively recovered from biological specimens using Dowex-1 columns. The procedure for digesting the protein and preparing the samples is given in detail. Recovery values for 72 samples using Co^{57} and Fe^{59} as tracers are tabulated. Excellent recovery and reproducibility are achieved.

1. Ogborn, R. E., Dunn, A. L., Blotcky, A. J., Johnson, G. F., James, L. R., *Am. J. Roentgenol.*, 1961, v85, 976.

2. Odeblad, E., Meurman, L., Ziliotto, D., *Acta Radiol.*, 1955, v44, 337.

3. Lowman, J. T., Krivit, W., *J. Lab. and Clin. Med.*, 1963, v61, 1042.

4. Kraus, K. A., Moore, G. E., *J. Am. Chem. Soc.*, 1953, v75, 1460.

Received April 29, 1963. P.S.E.B.M., 1963, v113.

Action of 5-Fluorodeoxyuridine on Synthesis of Infectious Canine Hepatitis Virus in Dog Kidney Cells.* (28488)

J. E. MOULTON AND L. M. FRAZIER
Department of Pathology, School of Veterinary Medicine, University of California, Davis

The DNA inhibitor, 5-fluorodeoxyuridine (FUDR)† interferes with the synthesis of thymine, probably at the level of thymidylate synthetase, and the inhibition is reversible with thymidine(1,2). FUDR or related compounds suppress synthesis of DNA viruses (3-7) but not RNA viruses(8-10). The effects of the drug on synthesis of infectious canine hepatitis (ICH) virus in dog kidney (DK) cells are described in this report. ICH virus, which is a member of the adenovirus group(11), multiplies in the nucleus where it stimulates formation of DNA(12,13).

Materials and methods. The procedures used in cultivation of DK cells and titration of ICH virus by the tube dilution method have been described(12). A 10^{-2} dilution of virus that titered at $10^{6.5}TCID_{50}$ per ml was used to infect the cultures and 2-hour adsorption periods were employed. The normal growth curve of the virus was determined by titrating at varying periods 2-42 hours after infection. The inhibitory effect of FUDR was studied by adding FUDR (10^{-3} M, 10^{-5} M, and 10^{-6} M) at varying periods 2-36 hours

* This work was supported in part by research grant from Nat. Inst. of Allergy and Infect. Dis., U. S. Public Health Service, Bethesda, Md.

† FUDR was kindly supplied by Dr. R. Duschinsky, Hoffmann-LaRoche Inc., Nutley, N. J.