When urine was obtained from the lambs, 24-hour fecal samples were also collected. Apparent N digestion (Table II), calculated by the chromic oxide ratio technic, was used as the denominator for correcting the respective creatinine N% values. This measurement was related to relative growth response of the lambs. Although metabolic fecal N was not obtained, this calculation stimulates the Thomas-Mitchell formula for determination of biological value. Both methods determine the comparative amount of absorbed N that is retained by the animal. While the Thomas-Mitchell method is most accurate for monogastric animals, the method proposed here provides a simple and practical procedure for determining comparative protein anabolism or biological value of protein in ruminant animals. Further, this method should be of particular value in determining relative duration of influences of relative magnitude of effects of growth-stimulating drugs.

Summary. A method for predicting comparative values of nitrogen sources and anabolic protein stimulation by drugs in ruminants has been proposed. The method requires determination of urinary creatinine N% divided by the apparent N digested (expressed as a decimal) in animals with similar N intake per unit body weight.

1. Mitchell, H. H., *J. Biol. Chem.,* 1924, v58, 873.
2. Harris, L. E., Mitchell, H. H., *J. Nutr.,* 1941, v22, 167.
3. Harris, L. E., Mitchell, H. H., *ibid.,* 1941, v22, 183.
4. Johnson, B. C., Hamilton, T. S., Mitchell, H. H., Robinson, W. B., *J. Anim. Sci.,* 1942, v1, 236.
5. Clegg, M. T., Albaugh, R., Lucas, J., Weir, M. C., *ibid.,* 1955, v14, 178.
6. Light, M. R., Dinusson, W. E., Richard, R. M., Bolin, D. W., *ibid.,* 1956, v15, 570.
7. Denning, J. S., Gallup, W. D., Briggs, H. M., *J. Biol. Chem.,* 1949, v177, 157.
8. Murlin, J. R., Hays, A. D., Johnson, K., *J. Nutr.,* 1953, v51, 149.

Received September 4, 1958. P.S.E.B.M., 1959, v100.

Rapid Method for Determination of Lipid Bound Radioiodine in Blood. (24662)

HERBERT D. STRAUSS AND HARRIET K. RICHARDS (Introduced by J. O. Lampen)
Veterans Administration Hospital, Philadelphia, Pa.

Studies of absorption of radioactive fats, and fatty acids, labeled with I^{131} have recently been shown(1,2,3) to provide useful clinical information in certain disorders of the gastrointestinal tract. Although much on radioactive fat absorption has described measurements on fecal collections(4), more recent studies have shown that measurements of radioactivity in blood could also be correlated with clinical status. For example, in idiopathic steatorrhea, pancreatitis, and pancreatic insufficiency, blood levels of radioactivity have been shown to be below values obtained with normal individuals. The ease with which blood radioactivity measurements could be made compared to data obtained from fecal collections, has also provided impetus for evaluation of blood radioactivity. In addition to measuring total I^{131} radioactivity in blood, some investigators(1,2,5) have determined amount of radioactivity in protein-bound form. One report(5) indicated that certain disease states, such as hypercholesteremia, lipemia and coronary artery disease are associated with alteration of ratio of lipid-bound radioiodine to inorganic radioiodine. Interest in protein-bound I^{131} measurements in fat absorption with I^{131} labeled fats, has led us to seek a more rapid procedure than the trichloroacetic acid precipitation of protein. Anion exchange resins, useful in removal of inorganic I^{131} in protein-bound "conversion ratio" studies(6,7,8) might also be suitable for separation of lipoprotein I^{131} radioactivity. If this separation could be performed on whole blood without prior re-

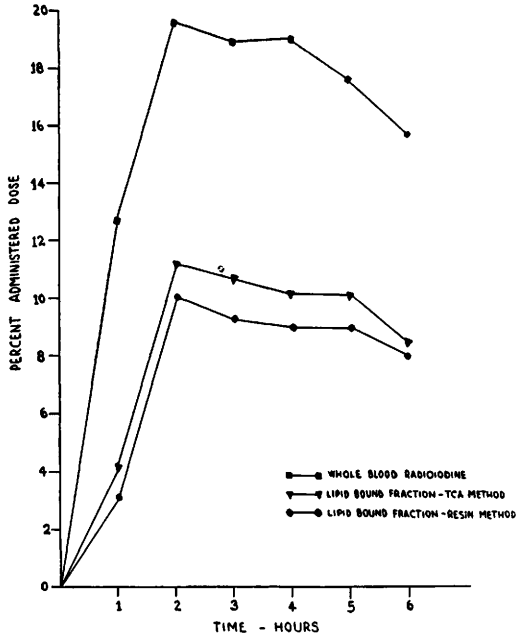

FIG. 1. Comparison of whole blood values and lipid-bound values done by TCA and Ion Exchange methods following a test dose of I^{131} labeled glyceryl trioleate.

moval of red blood cells, additional savings in time would result. Both of these objectives have been achieved through use of a new commercially available ion exchange kit.*

Materials and methods. A. *Trichloroacetic acid* (TCA) method. The procedure of Beres (1) and co-workers was followed with minor modification. B. *Ion exchange* method: Two ml of distilled water was added, with mixing, to 2 ml of heparinized radioactive whole blood in test tube. This mixture remained undisturbed 5 minutes to permit hemolysis of red blood cells. The red solution was now passed through the ion-exchange column. The column was then washed twice with 1 ml portions of isotonic saline to insure complete removal of protein-bound radioactivity from the column. These washes were added to original column eluate. All samples were counted in well-type scintillation counter. Since effluents from resin columns were not of equal volume and the well crystal is not linear for increasing volumes of fluid, it was necessary to make corrections in terms of counts observed versus volume of sample. A volume calibration curve was constructed for the well-counter so that all counting data could be corrected to the same volume. Eight subjects undergoing fat absorption studies with glyceryl trioleate were used. A total of 23 blood samples from these patients was used. Forty separate determinations were made with the TCA method, 14 of which were in duplicate. A regression analysis of the 23 pairs of percent TCA and percent resin determinations gives a correlation coefficient of 0.995 with a standard error of estimate of 0.44. Calculation of percent of administered dose present as lipid-bound radioiodine in whole blood is based on blood volume estimated as 75 ml blood/kg body weight. Therefore: % Lipid-bound radioiodine =

$$\frac{\text{Blood volume} \times \text{counts lipid-bound iodine/ml blood}}{\text{Counts original fat ingested}}$$

Results. These data show that both methods are reproducible to a high degree. In all cases, the resin method shows a slightly lower value than the TCA method. Nevertheless, similar curves may be obtained by serial determinations on the same subject, indicating that both technics are measuring the same entity (Fig. 1).

Summary. A method is reported for determination of lipid-bound radioiodine in blood by ion exchange resin technic. The method has been shown to be reproducible, accurate, rapid, and economical.

The authors wish to make acknowledgement to Mrs. Olha O. Holowecky for technical assistance, and Mrs. Winnie Strauss who prepared the graph.

* Rezikit® generously supplied by Dr. Paul Numerof of Squibb, New Brunswick, N. J.

1. Beres, P., Wenger, J., Kirsener, J. B., *Gastroenterol.*, 1957, v32, 1.

2. Berkowitz, D., Sklaroff, D., *Arch. Int. Med.*, 1957, v100, 951.

3. Duffy, B. J., Turner, D. A., *Ann. Int. Med.*, 1958, v98, 1.

4. Shingleton, W. W., Wells, M. H., Boylin, G. J., Ruffin, J. M., Sanders, A., *Surg.*, 1958, v38, 134.

5. Berkowitz, D., Sklaroff, D., Woldow, A., Jacobs, A. C., Likeff, W., *Clin. Research*, 1958, v6, 233.

6. Zieve, L., Vogel, W. C., Schultz, A. L., *J. Lab. and Clin. Med.*, 1956, v47, 663.

7. Scott, K., Reilly, W. A., *Metabolism*, 1954, v3, 506.

8. Fields, T., Kinnory, D. S., Kaplan, E., Oester, Y. T., Bowser, E. N., *J. Lab. and Clin. Med.*, 1956, v47, 333.

Received September 4, 1958. P.S.E.B.M., 1959, v100.

Incorporation of L-Carbamyl-C^{14}-Aspartate into Acid-Soluble Pyrimidine Nucleotides of Mouse Brain.* (24663)

WILLIAM T. MURAKAMI, DONALD W. VISSER, HAROLD E. PEARSON

Depts. of Biochemistry and Nutrition and Public Health, Univ. of Southern Calif. School of Medicine and Los Angeles County Hospital, Calif.

Our previous studies(1) showed that propagation of Theiler's GD VII strain of mouse encephalomyelitis virus in minced one-day-old mouse brain preparations results in increased uptake of P^{32} into ribonucleic acid phosphorus. Since it is known that acid-soluble nucleotides serve as precursors of cellular ribonucleic(2) and deoxyribonucleic acids(3), this study was undertaken to determine acid-soluble nucleotide constitution of noninfected and viral infected mouse brain and distribution of radioactivity in acid-soluble pyrimidine nucleotides and coenzymes following injection of a specific C^{14}-labeled pyrimidine nucleotide precursor, L-carbamyl-C^{14}-aspartate.

Methods. Synthesis of L-carbamyl-C^{14}-aspartate. L-carbamyl-C^{14}-aspartate was synthesized from urea-C^{14} (292 mg, specific activity 0.66 mc/mM), according to the method of Nyc and Mitchell(4). The alkaline reaction mixture was neutralized with concentrated HCl and the L-carbamyl-C^{14}-aspartate preparation was purified by separation on Dowex 1 (formate) X8 ion-exchange resin by the method of Lieberman and Kornberg (5). Based on the method of Koritz and Cohen(6) for determining carbamyl compounds, the specific activity of the final produce was 5.7×10^5 cpm/μmole. The calculated specific activity, based on urea, was 5.8×10^5 cpm/μmole. Although the L-carbamyl-C^{14}-aspartate solution was stored in deep-freeze, a chemical conversion to 5-carboxymethylhydantoin-C^{14} and to a smaller extent, dihydroorotate-C^{14} occurred. As much as 37% of total counts in the L-carbamyl-C^{14}-aspartate solution used for injection in Exp. 1 and 2 was present as 5-carboxymethylhydantoin. Chemical conversion was not detected when a neutralized L-carbamyl-C^{14}-aspartate solution was stored under similar conditions. *Injection of mice and preparation of acid-soluble fraction.* Mice, 5 to 6 weeks old, were injected intracranially in 6 sites with total of 0.024 ml L-carbamyl-C^{14}-aspartate solution (1×10^6 cpm/mouse). Mice were sacrificed 40 minutes after injection by cardiac exsanguination and brains were immediately dissected out and frozen by compression with dry ice. Frozen tissue was ground to a fine powder and homogenized with 2 volumes of ice-cold 0.6 N perchloric acid in tissue grinder, with a Teflon pestle. The homogenate was centrifuged and the protein pellet was extracted with 2 volumes of cold 0.2 N perchloric acid. Supernatant solutions from the 2 extractions were combined and perchloric acid was precipitated by addition of 5 N KOH to a phenol-red endpoint in an ice bath. After removal of $KClO_4$ by centrifugation, the perchloric acid-soluble fraction was chromatographed on a Dowex 1 (formate) X8 resin column. *Ion-exchange chromatography.* Purine and pyrimidine nucleotides present in perchloric acid-soluble extracts of mouse brain were separated by extended gradient elution chromatography system (modification of chromatographic system of Cohn(7) developed by Hurlbert *et al.* (8)). A more gradual formic acid gradient (mixer volume 1 liter, reservoir solution 2.3 N formic acid) was used for preliminary elution to get greater resolution of earlier

* Aided by grant from Nat. Inst. of Health and Am. Cancer Society.