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Bile acids
[11, 12-^{2}H]chenodeoxychloic acid

isotope dilution
stable isotope

Validation of Use of 11,12-^{2}H-Labeled Chenodeoxycholic Acid in Isotope Dilution Measurements of Bile Acid Kinetics in Man

WILLIAM F. BALISTRERI, ALISTAIR E. COWEN, ALAN F. HOFMANN,[31] PATRICIA A. SZCZEPANIK, AND PETER D. KLEIN

Gastroenterology Unit, Mayo Clinic and Mayo Foundation, Rochester, Minnesota, and the Argonne National Laboratory, Argonne, Illinois, USA

Extract

Chenodeoxycholic acid labeled with ^{2}H in the 11 and 12 positions was prepared by catalytic reduction of $\Delta^{11\text{-}12}$ unsaturated derivatives of cholic acid. To validate the use of this stable isotope for the determination of bile acid kinetics by isotope dilution, it was administered to seven normal male volunteers simultaneously with [24-^{14}C]chenodeoxycholic acid. Bile was collected at regular intervals over the following 5 days, and the chenodeoxycholic acid pool size and fractional turnover rate were determined from the specific activity decay curve for ^{14}C and from the isotopic abundance curve for ^{2}H. Estimates of the pool size by both isotopes showed a correlation of $r = 0.95$ and similar precision. Synthesis rate, the product of pool size and fractional turnover rate, also showed good agreement ($r = 0.97$), Because previous investigations have shown that bile acids tagged with hydrogen isotopes at the 11 and 12 positions are stable in man, the present data suggest that 11,12-^{2}H-labeled bile acids may be used in place of radioactive isotopes for valid isotopic measurement of bile acid kinetics in healthy infants and children.

Speculation

The availability of bile acids labeled with stable isotopes, for example, ^{2}H or ^{13}C, would permit bile acid metabolism to be studied by isotope dilution measurements in any population of patients without radiation hazard.

Measurement of bile acid pool size and synthesis rate by the technique of isotope dilution has become a standard practice in gastroenterology (6). Since the initial description of the method by Lindstedt (13), isotope dilution has provided useful information on bile acid metabolism both in health (2, 19) and in such diseases as cholelithiasis (17), cirrhosis (18), hyperlipidemia (9, 11), and ileal dysfunction (4). However, use of this technique requires the administration of a labeled bile acid. Commonly, bile acids labeled with ^{14}C in the carboxyl group or with ^{3}H in the nucleus have been used, but administration of such bile acids entails a radiation hazard, and this has precluded their use in healthy children. Admittedly, the radiation exposure associated with the administration of ^{14}C- or ^{3}H-labeled bile acids is small because the biologic half-life is less than 1 week (19) and the anatomic distribution, at least in health, is limited to the enterohepatic circulation. Nonetheless, the ready availability of a primary bile acid labeled with a stable isotope possessing stability *in vivo* would eliminate even this small risk and allow investigation of bile acid metabolism in children in health and disease.

Our laboratory has recently reported a rapid and simple method for preparing 11,12-^{2}H-labeled chenodeoxycholic and lithocholic acids by heterogeneous catalytic reduction of their $\Delta^{11,12}$ derivatives (1). We showed further, by using 11,12-^{3}H-labeled bile acids, that in healthy subjects this label is stable during enterohepatic cycling, including exposure to the enteric flora. To use 11,12-^{2}H-labeled bile acids for isotope dilution studies, precise mass spectrometric techniques for quantifying the proportion of di-deutero bile acids in bile acid samples are required. These have been developed previously (10), validated (5), and used for limited clinical studies (20, 21) with 2,4-^{2}H-labeled bile acids (3).

In this paper we report experiments aimed at validating use of 11,12-^{2}H-labeled bile acids for isotope dilution studies of bile acid metabolism in man.

METHODS

LABELED BILE ACIDS

[11,12-^{2}H]Chenodeoxycholic acid was synthesized by Merck, Sharp and Dohme (22) by catalytic reduction of 3α,7α-dihydroxy chol-11,12-ene-5β-oic acid with deuterium gas as described recently (1). The compound was purified by column chromatography and its purity confirmed by thin layer chromatography; isotope content was established by mass spectrometry (10). [24-^{14}C]Chenodeoxycholic acid was purchased from New England Nuclear (23), and found to have a radiopurity of greater than 95% by zonal scanning (16).

EXPERIMENTAL DESIGN

The study was carried out in seven healthy male volunteers, ages 20–29 years, from whom informed consent was obtained. After overnight fasting, each subject was given 10 μCi [24-^{14}C]chenodeoxycholate and 38 mg [11,12-^{2}H]chenodeoxycholate simultaneously by intravenous injection at 0800. On the following morning, a single lumen nasoduodenal tube was positioned using fluoroscopy with image intensification; the aspiration site was distal to the ampulla of Vater. Gall bladder contraction was stimulated by intravenous injection of cholecystokinin-pancreozymin (75 units) obtained from Karolinska Institutet (24). Approximately 30 ml bile were aspirated. The aspirated bile was mixed, a 3-ml sample was removed, and the remainder of the bile was injected back into the tube. Duodenal aspiration was repeated at 1600 and 0800 for the next 2 days. Six separate samples were obtained for calculations of specific activity and isotopic abundance.

Subjects kept the nasoduodenal tube in place throughout the study; they were studied as outpatients, and they engaged in their normal physical activity and ate a diet of their own choice.

ANALYSIS

Mass Determination of Chenodeoxycholic Acid. The 3-ml sample of bile was mixed with 15 ml of isopropanol and kept at 5°

before analysis. Two 1-ml aliquots of this isopropanol solution were taken for duplicate determination of total bile acid concentration by an automated modification of the steroid dehydrogenase method of Iwata and Yamasaki (8). Relative biliary bile acid composition was estimated by taking a 6-ml aliquot of each sample for gas-liquid chromatography of the methyl ester acetates as described previously (7, 15). Total chenodeoxycholic acid mass (micromoles per ml) was calculated from total bile acid mass and relative percentage of chenodeoxycholic acid.

Deuterium Determination. An aliquot of the solution of the methyl ester acetates was applied to a thin layer chromatographic plate which then was developed with hexane-acetone, 70:30 (v/v). The band with the mobility of dihydroxy bile acids was scraped off and eluted. The solvent was removed and these samples were analyzed at Argonne National Laboratories. The isotopic content of [11,12-$^2H_2$]chenodeoxycholic acid in each sample was determined by gas chromatography-mass spectrometry using the alternating voltage acceleration technique described previously (10). Standards of known concentration (1:20) were analyzed with each series of samples to provide internal references of isotopic content.

^{14}C Specific Activity. Because lithocholic acid constituted less than 2% of the total bile acids after alkaline saponification, radioactivity measurements of ^{14}C were made directly on the isopropanol solution of bile. Duplicate 500-μl samples were dried, reconstituted in an equal volume of 0.05 N sodium hydroxide, decolorized under ultraviolet light, and dispersed in 14 ml toluene-based scintillation cocktail (Ready-Solv 6 (25)). ^{14}C radioactivity was determined by liquid scintillation spectroscopy using external standardization for quench correction.

Calculation of Bile Acid Kinetics. Bile acid kinetics were calculated as described previously (2, 4). The coefficient of variation of the curves obtained was less than 8%. For estimation of pool size and turnover rate from ^2H, corrections were made for the amount of ^2H administered by subtracting the quantity administered from the final pool size calculated.

RESULTS

Pool size, synthesis rate, and fractional turnover rate measurements using these two isotopes show excellent correlation and an acceptable agreement in absolute values (Table 1 and Figs. 1 and 2). The pool size measurements (r = 0.955) were an average of 10% higher using the ^2H chenodeoxycholic acid, and the fractional turnover rates (r = 0.917) were also higher, averaging 117% of the ^{14}C values. The synthesis rates, which were highly correlated (r = 0.971), reflected the cumulative effects of pool size and fractional turnover rate increases to result in synthesis rates that were 26.8% higher in the studies with ^2H than with ^{14}C. In all three correlations, the intercepts were not significantly different from 0. Of the three slope measurements, only the synthesis rate showed a statistically significant difference between isotopic forms.

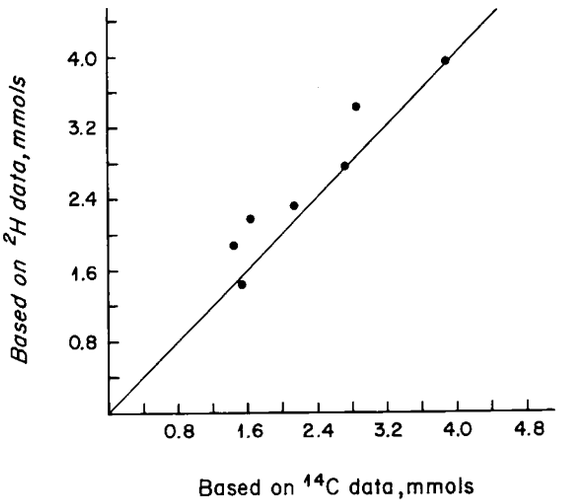

Fig. 1. Comparison of chenodeoxycholic acid pool size when estimated using [24-^{14}C]chenodeoxycholic acid (*abscissa*) or simultaneously administered [11,12-^2H]chenodeoxycholic acid (*ordinate*).

The precision of the methods was similar, with a mean coefficient of variation of duplicates of 8.3% for ^{14}C and 7.6% for ^2H. A typical curve for ^{14}C and ^2H is shown in Figure 3.

DISCUSSION

These data indicate the validity of pool size and synthesis rate measurements of chenodeoxycholic acid using [11,12-^2H]-chenodeoxycholic acid. The observed differences in synthesis rate are, we suspect, in all likelihood due to methodologic imprecisions associated with the ^{14}C studies rather than an isotopic effect manifested by the ^2H form. The rationale for such a choice lies in the departure from our usual procedures for mass determinations of chenodeoxycholic acid in this study. Total bile acid was determined by steroid dehydrogenase assays on the total bile acid sample before fractionation, instead of on the dihydroxy bile fraction, which is our usual practice. By contrast, the proportion of ^2H and ^1H chenodeoxycholic acid is determined within the same measurement and is not influenced by procedural losses during purification. Moreover, an isotopic effect can only be manifested

Table 1. *Comparison of chenodeoxycholate pool size and synthesis rate measured with ^{14}C or ^2H*[1]

	Pool size, mmol			Fractional turnover rate, days^{-1}			Synthesis rate, mmol/day		
Subject	^{14}C	^2H	Difference, %	^{14}C	^2H	Difference, %	^{14}C	^2H	Difference, %
1	1.55	1.43	−8.1	0.38	0.46	+19.0	0.58	0.65	+11.4
2	1.45	1.88	+25.8	0.33	0.31	−6.3	0.47	0.58	+21.0
3	1.62	2.16	+28.6	0.20	0.19	−5.1	0.33	0.39	+16.7
4	2.73	2.77	+1.5	0.11	0.16	+37.0	0.30	0.43	+35.6
5	2.89	3.42	+16.8	0.10	0.13	+26.0	0.27	0.40	+38.3
6	2.14	2.31	+7.7	0.23	0.25	+8.3	0.49	0.62	+23.4
7	3.89	3.92	+0.7	0.24	0.35	+37.3	0.92	1.40	+41.3
Mean ± SE	2.32±0.34	2.56±0.33	+10.4±5.2	0.23±0.04	0.26±0.04	+16.6±6.9	0.48±0.08	0.64±0.13	+26.8±4.0

[1] Regression analysis, ^{14}C vs ^2Hv.

Intercept	0.391±0.319	0.029±0.049	−0.093±0.088
Slope	0.931±0.130	1.035±0.201	1.524±0.168
Correlation coefficient	0.954	0.917	0.971

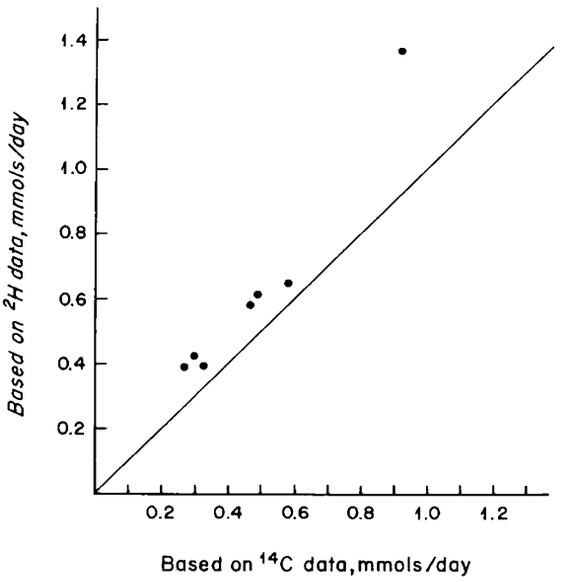

Fig. 2. Comparison of chenodeoxycholic acid synthesis rate, when estimated using [24-^{14}C]chenodeoxycholic acid (*abscissa*) or [11,12-^{2}H]-chenodeoxycholic acid (*ordinate*).

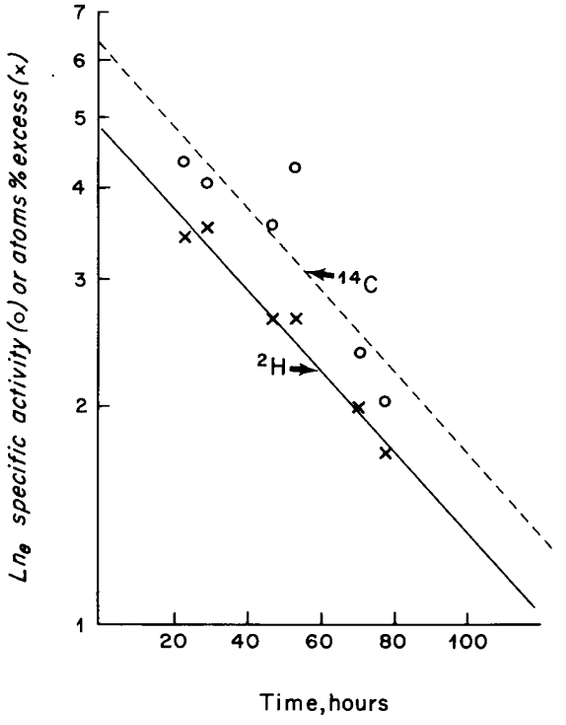

Fig. 3. Representative specific activity decay curve (O) or atoms percent excess decay curve (×) after administration of labeled chenodeoxycholic acid.

during processes involving reactivity of the labeled site and, in this regard, the labeled position appears to be inert during the enterohepatic circulation.

Although the 2,4-^{2}H-labeled bile acids previously synthesized by us (3) and used for kinetic studies in children by Watkins *et al.* (20, 21) are suitable for measurements of chenodeoxycholic acid kinetics *per se*, the lability of the label after its loss from the enterohepatic circulation in the intestinal tract limits studies of bile acid interconversions with this product (12, 14). The development of the [11,12-^{2}H]chenodeoxycholic acid is therefore a twofold improvement: the 11,12 label is simpler to introduce into the molecule in a predictable manner, and it exhibits true biologic stability *in vivo*.

SUMMARY

Chenodeoxycholic acid labeled with ^{2}H in the 11 and 12 positions was prepared by catalytic reduction of $\Delta^{11\text{-}12}$ unsaturated derivatives of cholic acid. To validate the use of this stable isotope for the determination of bile acid kinetics by isotope dilution, it was administered to seven normal male volunteers simultaneously with [24-^{14}C]chenodeoxycholic acid. Bile was collected at regular intervals over the following 5 days, and the chenodeoxycholic acid pool size and fractional turnover rate were determined from the specific activity decay curve for ^{14}C and from the isotopic abundance curve for ^{2}H. Estimates of the pool size by both isotopes showed a correlation of $r = 0.95$ and similar precision. Synthesis rate, the product of pool size and fractional turnover rate, also showed good agreement ($r = 0.97$). Because previous investigations have shown that bile acids tagged with hydrogen isotopes at the 11 and 12 positions are stable in man, the present data suggest that 11,12-^{2}H-labeled bile acids may be used in place of radioactive isotopes for valid isotopic measurement of bile acid kinetics in healthy infants and children.

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22. Montreal, Quebec, Canada.
23. Boston, Mass.
24. Stockholm, Sweden.
25. Beckman, Fullerton, Calif.
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27. Dr. W. F. Balistreri is a Research Fellow, Children's Hospital Research Foundation, University of Cincinnati, Ohio.
28. Dr. A. E. Cowen is an Eli Lilly Fellow. Present address: Department of Medicine, Royal Brisbane Hospital, Australia.
29. The present address of Drs. P. A. Szczepanik and P. D. Klein is: Division of Biological and Medical Research, Argonne National Laboratory.
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31. Requests for reprints should be addressed to: A. F. Hofmann, M.D., Mayo Clinic, Rochester, Minn. 55901 (USA).
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Corticotropin lung
fetus saline
glucocorticoids

Pulmonary Pressure-Volume Relationships after Corticotropin (ACTH) and Saline Injections in Fetal Rabbits

MARIE F. ROBERT, ANDREW T. BATOR, AND H. WILLIAM TAEUSCH, JR.[24]

McGill University-Montreal Children's Research Institute, and the Department of Physiology, McGill University, Montreal, Quebec, Canada

Extract

ACTH mixed with barium sulfate injected into fetal rabbits on *day 24* of a 31-day gestation was associated with increased lung maturity compared with lungs of noninjected controls when assessed by deflation pressure–volume curves and lung weight to body weight ratios measured on *day 27*. Fetuses injected with saline and barium sulfate also had accelerated (although somewhat less) lung maturation, perhaps from an ACTH-mediated response to stress. The ACTH group (*A*) maintained the largest lung volumes on deflation, followed by the saline group (*B*), the ACTH controls (*C*), and the saline controls (*D*). Volumes (percentage of total lung capacity) between the two injected groups and their controls (*A* versus *C; B* versus *D*) were significantly different at transthoracic pressures of 15, 10, 7, 4, 0, and −2 cm water ($p < 0.05$). Volumes (percentage of total lung capacity) between the ACTH group and the saline group (*A* versus *B*) and between the two controls groups (*C* versus *D*) were not significantly different. Wet lung weight to body weight ratios were significantly different between the injected groups and their respective controls, between the two injected groups, and between the two control groups.

Speculation

Changes in pressure-volume relationships and lung weight to body weight ratios after injections of ACTH or saline into fetal rabbits support the hypothesis that fetal glucocorticoids are physiologic regulators of lung maturation.

In 1959, Avery and Mead (2) established that hyaline membrane disease is associated with inadequate amounts of surfactant in the premature lung. Pharmacologic means of inducing the fetal lung to produce surfactant precociously have been reported (17–19). In particular, glucocorticoids, in pharmacologic amounts, have been shown to accelerate lung maturation in various animal models (7, 11), and have reduced the incidence of hyaline membrane disease in human infants treated prenatally (9). Sundell *et al.* (15) have found evidence of increased lung maturity and decreased hyaline membrane disease in lambs treated with continuous infusion of ACTH for 5 days before premature delivery. The aim of our study was to investigate further the effects of ACTH on lung maturation in fetal rabbits.

MATERIALS AND METHODS

Twenty-three white New Zealand rabbits were mated so that the onset of pregnancy was known to within 1 hr (20). On *day 24* (1 week before term), laparotomies were performed on pregnant does anesthetized with a halothane-oxygen mixture after intubation facilitated by exposure to ether. Three to five fetuses in each litter were injected intramuscularly (through the uterine wall), either with 0.25 or 1.00 mg ACTH (21) mixed in saline with 15 mg barium sulfate or with an equal volume (0.1 ml) of saline and barium sulfate. These ACTH doses were chosen because they were found to be in excess of quantities sufficient to cause increases in peripheral blood levels of glucocorticoids in term newborn rabbits (16). Differences between fetuses treated with low or high doses of ACTH were not detected. Therefore, ACTH-injected fetuses are considered as a single group. Members of 10 litters received ACTH (and barium sulfate) with their noninjected littermates as controls; members of the other 13 litters received saline (and barium sulfate) with similar littermate controls. For purposes of comparison, the groups are labeled: *group A*, ACTH injected; *group B, saline injected; group C*, ACTH littermates; and *group D*, saline littermates. Experimental procedures were performed under sterile conditions. Barium sulfate, visible on radiograph, served as a marker to confirm that the injection entered the fetuses (4).

On *day 27* (72 hr ± 8 hr after injection), the does were killed