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EVALUATION OF TRITIUM CHOLESTEROL AS A TRACER IN MAN*

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INTRODUCTION

STUDIES have been in progress during the past three years in this laboratory[1] to determine the percentage of the urinary steroids in man that is derived from cholesterol. A part of this project has involved the feeding of tritium-cholesterol (cholesterol-t) to human beings for extended periods of time, *e.g.*, as long as two weeks, and the isolation of labelled plasma free cholesterol and urinary steroids. Before these studies were undertaken, it was necessary to establish 1) that the tritium in cholesterol-t, prepared by catalytic exchange, was not labile in the course of the catabolism of the sterol in the body to bile acids and steroids; and 2) that tritium derived from the degradation of the side chain did not re-enter newly synthesized cholesterol or steroid hormones to any appreciable extent. Therefore an investigation was initiated to evaluate the use of cholesterol-t as a tracer in man. This paper presents the data that were obtained from this study.

The distribution of tritium, between the side chain and the tetracyclic

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rings, was established by chemical degradative procedures. A male subject was given intravenously a mixture of carbon-14 and tritium cholesterols of known specific activities. Plasma free cholesterol and five urinary steroids were isolated, and the ratio of tritium to carbon-14 specific activities was determined by means of a liquid scintillation counter. The findings demonstrated that exogenous cholesterol was a precursor of three urinary adrenal steroid metabolites namely, pregnan-3-α-17-α-21-triol-11,20-dione (THE), pregnan-3-α-11-β-17-α-21-tetrol-20-one (THF), and etiocholan-3-α-ol-11, 17-dione (11-ketoetiocholanolone) *in vivo* (1, 2). The data also showed that cholesterol-t could be used as a tracer in man for catabolic studies involving that sterol. The advantages of using tritium in place of carbon-14 as a tracer for investigations in human beings have been described by Biggs and Kritchevsky (3).

MATERIALS AND METHODS

All melting points were taken on a Fisher-Johns apparatus.

Tracers: Cholesterol-t was prepared by an exchange reaction with tritium water (THO) (4, 5). Labile tritium was removed by refluxing the cholesterol for two hours with 5 per cent potassium hydroxide in methanol (6). After 2 crystallizations from 80 per cent alcohol, the cholesterol-t had a melting point of 146–147°, and a specific activity (s.a.) of 7.3 μc. per mg. The 4-C^{14}-cholesterol (m.p. 147–148°, s.a. 5.0 μc. per mg.) was obtained from Radioactive Products Inc., Detroit, Michigan.

Patient and administration of cholesterol: The patient was a 60-year-old white man with chronic rheumatoid arthritis. Cholesterol-t (33.8 μc.) and 4-C^{14}-cholesterol (4.24 μc.) were dissolved in 2.3 Gm. of propylene glycol. The solution was dispersed in 30 ml. of sterile human plasma that was administered intravenously with no untoward effects in a single dose.

Distribution of tritium in cholesterol: Cholesterol-t was diluted with "cold" cholesterol and converted to cholesteryl chloride-t by the method of Blecher and Gurin (7). The steroid melted at 97° after purification by chromatography on aluminum oxide (8) and crystallization from acetone-alcohol. A 3-Gm. portion was pyrolyzed by the procedure of Bloch and Rittenberg (8). After pyrolysis, the mixture of iso-octane and iso-octene was distilled off. The residue was chromatographed on aluminum oxide, and the $C_{19}H_{30}$ hydrocarbon was eluted from the column and counted.

$C_{19}H_{30}$—calculated, C 88.4, H 11.6; found, C 88.4, H 11.3

One gram of cholesterol-t was subjected to the Oppenauer oxidation as described by Jones *et al.* (9). Following chromatography and crystallization from acetone, the Δ^4-cholestenone-t melted at 87.5–88°. The steroid was refluxed with aqueous methanolic potassium hydroxide under nitrogen for two hours, and after re-isolation was found to have the same melting point.

A 12-Gm. portion of cholesterol-t was converted to methyl-3β-acetoxy-Δ^5-cholenate-t by the procedure of Fukushima and Gallagher (4). After repeated crystallization from acetone-methanol, the steroid melted at 157.2–158.6° (156.5–157.5° reported).

Isolation of plasma free cholesterol: The method for the isolation and radioassay of plasma cholesterol has been described (10).

Hydrolysis and extraction of
to pH 6.5 and 75 units/ml. of
for forty-eight hours at 37° and
the urine was extracted conti
steroid extract was prepared a

Isolation and identification
of Burton *et al.* (12) and Savard
of THE from THF were obtai
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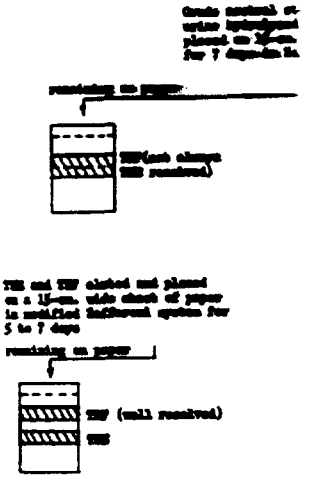
FIG. 1. FLOW SHEET F



steroids. THE and THF were
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The C_{19} steroids were ident
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was converted to the acetate
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were studied. At this point, ca
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In the case of etiocholan-3-α-
sample containing 36 per cent

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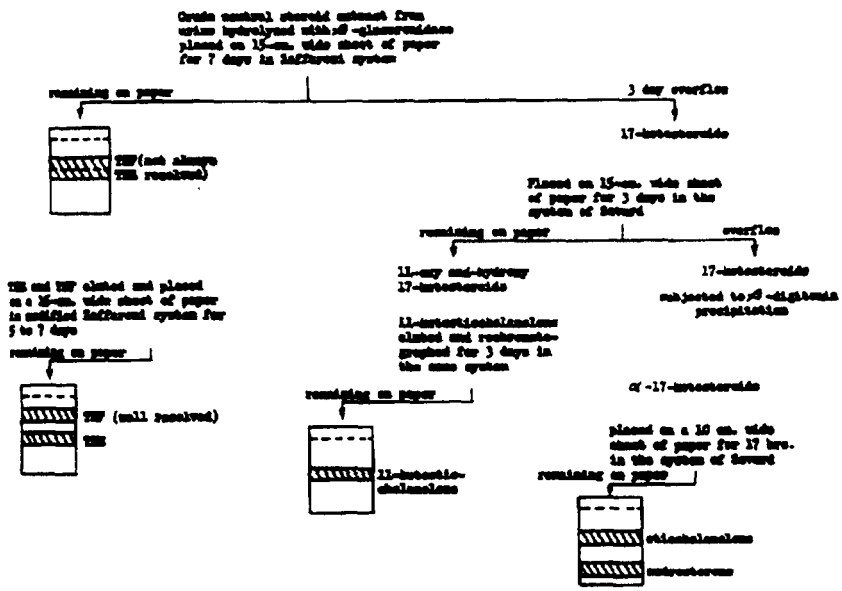
Hydrolysis and extraction of the urine: Twenty-four-hour urine samples were adjusted to pH 6.5 and 75 units/ml. of β-glucuronidase (Sigma) was added. Following incubation for forty-eight hours at 37° and the addition of 90 Gm./L. of anhydrous sodium sulphate, the urine was extracted continuously with ether for two days, and a crude neutral steroid extract was prepared according to the method of Baggett *et al.* (11).

Isolation and identification of urinary steroids: The paper chromatographic methods of Burton *et al.* (12) and Savard (13) were used to separate the steroids. Good separations of THE from THF were obtained when the Whatman #1 paper was impregnated with a solution containing 2 parts of methanol to 1 part of propylene glycol prior to application of the crude steroid mixture. Figure 1 is a flow sheet for the separation of the

FIG. 1. FLOW SHEET FOR THE SEPARATION OF THE URINARY STEROIDS.



steroids. THE and THF were assayed by the method of Chen *et al.* (14) and 17-ketosteroids were determined by the procedure described by Callow *et al.* (15), using stabilized alcoholic potassium hydroxide (16).

The C_{19} steroids were identified by paper chromatography in two solvent systems (ligroin-propylene glycol and ligroin-phenylcellosolve). An aliquot of each eluted steroid was converted to the acetate and was identified by paper chromatography (ligroin-formamide). Initial identification was assumed if the unknown, the authentic steroid, and the mixture had the same R_f values in all the paper chromatographic systems that were studied. At this point, carrier steroid was added and after several crystallizations, if the specific activity remained constant, identification was considered complete—provided the melting point of the steroid agreed with that recorded in the literature. In the case of etiocholan-3-α-ol-17-one (etiocholanolone), the infrared spectrum of a sample containing 36 per cent urinary steroid was identical with the spectrum of au-

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thentic etiocholan-3-α-ol-17-one. 11-Ketoetiocholanolone was isolated and counted as the acetate.

The procedure for the isolation and identification of THE has been described (1). Part of a pooled sample of THF was converted to the diacetate and was crystallized from methanol without the addition of carrier steroid. The identification of THF was carried out in the same manner as that described for THE (1). The ultraviolet spectrum of the THF diacetate after two hours in concentrated sulphuric acid was identical with that of the spectrum of pure THF diacetate. Carrier was added to the remainder of the pooled THF diacetate, and the mixture was crystallized from methanol-ligroin (1:1). The infrared spectrum of the crystalline material (70 per cent urinary steroid, 30 per cent carrier) coincided with the spectrum of authentic THF diacetate.

Radioassay: The steroid was dissolved in 50 ml. of toluene containing 100 mg. of 2,5-diphenyloxazole in a tall form-weighing bottle (17). The solution was cooled to −20° and counted in a Packard liquid scintillation counter, purchased from the Packard Instrument Co., La Grange, Ill. The carbon-14 was assayed by screening out the tritium pulses by a proper choice of discriminator settings. Then after resetting the discriminators, both the carbon-14 and tritium counts were registered simultaneously. The following equation was employed to calculate the carbon-14 and tritium disintegrations per minute:

$$\text{DPM } C^{14} = \frac{\text{cpm } C^{14}\ (B)}{C^{14} \text{ efficiency } (B)}$$

$$\text{DPM T} = \frac{\text{cpm } (T + C^{14})\ (A) - \text{DPM } C^{14} \times \text{efficiency } C^{14}\ (A)}{\text{T efficiency } (A)}.$$

A and B were two discriminator settings. At setting B the tritium counts were screened out effectively. Table 1 shows the accuracy attained by this method of counting at the levels of carbon-14 and tritium that were found in the isolated steroids. The time of counting was sufficiently long so that the probable error of counting was no greater than ±5 per cent.

After counting, the steroid was reisolated chromatographically on aluminum oxide or silica gel. The scintillator was eluted with benzene, and the steroid was eluted by increasing the polarity of the benzene with alcohol.

Five milligrams of THE diacetate had no quenching effect on the carbon or tritium that was counted. It was assumed that the other steroids behaved similarly.

RESULTS AND DISCUSSION

Figure 2 shows the distribution of tritium in cholesterol-t as determined by several degradative procedures. The distribution of the tritium between the side chain and the tetracyclic rings is similar to that found by Fukushima and Gallagher (4) for deuterated cholesterol. Thus, 47.2 per cent of the tritium was located on carbon atoms 24, 25, 26 and 27. (The deuterated steroid had 52 per cent of the total radioactivity at these positions (4).) There was no tritium in the hydroxyl group because the cholesteryl chloride-t had the same specific activity as the cholesterol-t from which it was prepared. Positions 2, 3, 4 and 6 had 37.5 per cent of the tritium. (The deuterated cholesterol had 40 per cent and 3 per cent of the deuterium on carbon atoms 6 and 3, respectively (4).) Since the $C_{18}H_{38}$-t hydrocarbon and

TABLE 1.

T DPM	
736	
831	
903	
996	
Av. 886	Me:

T DPM	
327	
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460	
571	
Av. 448	Me:

* The same amo

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TABLE 1. COUNTING ERRORS FOR TRITIUM AND CARBON-14 MIXTURES

Mixture prepared* DPM T 792 DPM C^{14} 342 Ratio T/C^{14} = 3.26

Found

T DPM	% Error	C^{14} DPM	% Error	Ratio T/C^{14}	% Error
736	7.1	244	0.4	2.77	15.0
831	4.9	266	9.5	3.09	5.2
903	14.0	266	9.5	3.39	4.0
996	25.7	269	10.7	4.08	25.2
Av. 886	Mean 12.9	Av. 261	Mean 7.5	Av. 3.31	Mean 12.4

Mixture prepared* DPM T 396 DPM C^{14} 121 Ratio T/C^{14} = .327

Found

T DPM	% Error	C^{14} DPM	T Error	Ratio T/C14	% Error
327	13.4	130	7.4	2.50	23.5
423	9.3	131	8.3	2.97	9.2
460	16.2	136	12.4	3.40	4.0
571	44.1	146	20.7	4.39	34.2
Av. 448	Mean 21.8	Av. 136	Mean 12.2	A. 3.31	Mean 17.7

* The same amounts of tritium and carbon-14 in four weighing bottles.

the methyl-3-β-Δ^5-cholenate-t had the same specific activity within the experimental error (Fig. 2), it was concluded that tritium was not stably bound at carbon atoms 20, 21, 22 and 23. About 16.2 per cent of the tritium in the rings was located at undetermined positions.

Table 2 shows the ratios of the tritium to carbon-14 specific activities of the plasma free cholesterol that was isolated during the ten-day period following administration of the tracers. The T/C^{14} ratio of the mixture of cholesterols that was administered was 8.0. The average value of the ratio for the blood cholesterol that was isolated was 7.0 ± 1.0. The 13 per cent discrepancy is probably not significant in view of the mean error of 12–17 per cent in determining the ratios of T/C^{14} (Table 1). These data indicate that cholesterol-t is stable in the blood for at least ten days. A similar conclusion was reached by Biggs and Kritchevsky (3) from studies of rabbits.

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TABLE 2. STABILITY OF CHOLESTEROL-T *in vivo*

Time after administration of tracers (days)	Plasma free cholesterol		Ratio of specific activities
	μc.T/mM	μc.C^{14}/mM	T/C^{14}
0.17	0.44	0.65	6.7
0.33	0.24	0.031	7.7
0.50	0.30	0.034	8.8
2	0.19	0.029	6.6
3	0.30	0.053	5.7
4	0.26	0.039	6.8
6	0.20	0.025	8.0
8	0.12	0.017	6.8
10	0.12	0.019	6.3

The carbon-14 and tritium specific activities of the three C_{19} and two C_{21} urinary steroids that were isolated in this study are presented in Table 3. The ratios of the T/C^{14} specific activities are shown in the last column. The mean value for all the ratios was 4.0 ± 0.9.

By multiplying the percentage of tritium found in the rings of cholesterol-t (53.2 per cent) by the mean value of the T/C^{14} ratio of the plasma free cholesterol, one obtains 3.8, the value expected for the T/C^{14} ratio of the urinary steroids. If 8.0, the value of the ratio of specific activities of the

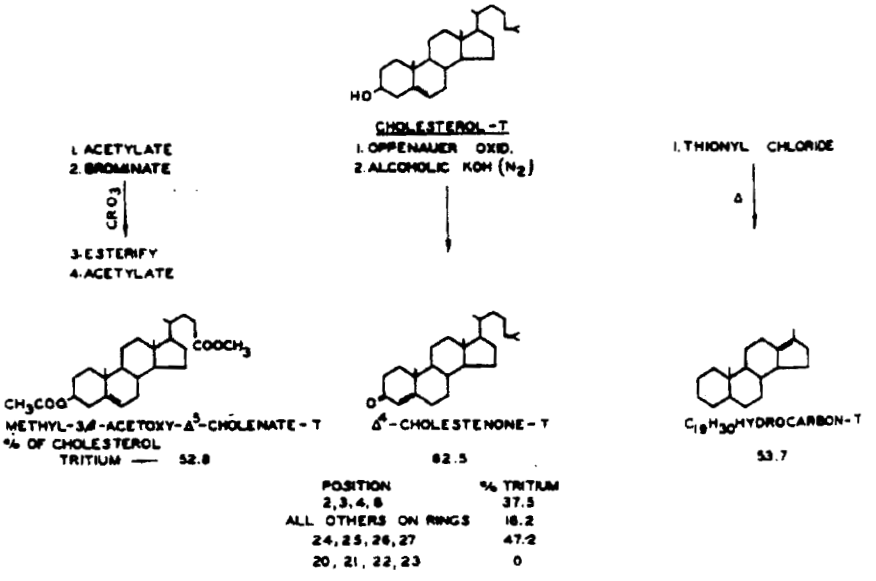

FIG. 2. Distribution of tritium in cholesterol-t.

TABLE 3. SPE

Day urine collected	Amt. isolated from urine (mg.)
1	1.75
4	1.24
5+6	2.40
7+8	1.50
1+2	3.18
4	1.90
5+6	4.98
1+8	1.67
2	3.46
4	2.39
5+6	4.60
2+6	1.1
	7.64

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TABLE 3. SPECIFIC ACTIVITIES OF CRYSTALLINE STEROIDS ISOLATED FROM THE URINE BY CARRIER TECHNIQUE

Day urine collected	Amt. isolated from urine (mg.)	Carrier added (mg.)	Steroid counted		Calculated by isotopic dilution		Ratio of specific activities T/C[14]
			m.p.° C	mg.	μc/C mM	μc/C[14] mM	
Androstan-3-α-ol-17-one							
1	1.75	17.59	186	10.77	0.11	0.21	5.2
4	1.24	10.44	185	8.30	0.089	0.035	2.5
5+6	2.40	7.91	186.5	1.82	0.10	0.029	3.5
7+8	1.50	10.14	186–187	6.38	0.072	0.022	3.4
Etiocholan-3-α-ol-17-one							
1+2	3.18	8.60	151–152	8.05	0.078	0.020	4.0
4	1.90	5.00	151–152	4.04	0.10	0.027	3.6
5+6	4.92	8.72	150–151	5.25	0.12	0.025	5.3
Etiocholan-3-α-ol-11,17-dione Acetate							
1+8	1.67	9.91	161–164	6.20	0.084	0.028	3.0
Pregnan-3-α-17-α-21-triol-11,20-dione Diacetate							
2	3.46	10.09	232–233.5	5.01	0.087	0.017	5.2
4	2.39	7.89	234	7.06	0.062	0.024	3.9
5+6	4.60	4.87	232.5	3.73	0.099	0.027	3.6
Pregnan-3-α-11-β-17-α-21-tetrol-20-one Diacetate							
2+6	1.1	—	209–217	1.1	0.11	0.021	5.2
	7.64	3.75	213–222	2.69	0.12	0.024	4.9

administered cholesterols, be used, then the expected ratio for the urinary steroids is 4.3. These calculations are based on the following assumptions: 1) that the conversion of cholesterol-t to urinary steroids involves no loss of tritium from the rings; 2) that the tritium from positions 24, 25, 26 and 27 on the side chain is not converted to urinary steroids to any appreciable extent; and 3) that the tetracyclic rings of 4-C^{14}-cholesterol are not degraded to smaller fragments, thereby releasing carbon-14 for possible steroid synthesis.[2]

The difference between the calculated values for the T/C^{14} ratio and that actually found in the urinary steroids was about 7.5 per cent. In view of the errors involved in determining the ratios, the agreement between the expected and found values is good and validates the assumptions made above.

[2] The authors are indebted to Dr. George Okita who assayed the expired air of the subject at intervals following administration of the labeled cholesterols. No $C^{14}O_2$ was detected. The lower limit of the assay was 1.8×10^{-4} μc./mM of $C^{14}O_2$. This indicates that degradation of the ring structure of carbon-14 cholesterol is unlikely.

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The results of adrenal perfusion (18) and adrenal homogenate (19, 20) experiments have shown that cholesterol can serve as a precursor of cortisol. The data in Table 3 demonstrate that this conversion can take place *in vivo*, since cortisol has been isolated from adrenal vein blood (21, 22) and THE, THF and 11-ketoetiocholanolone are metabolites of this steroid (23).

Ungar and Dorfman (24) isolated carbon-14 androsterone and etiocholanolone from the urine of a female patient with an adrenocortical carcinoma who had been fed 3-C^{14}-cholesterol. The isolation of labeled androsterone and etiocholanolone in the present study suggests that under normal physiologic conditions, cholesterol is also a precursor for the 11-desoxy-C_{19}-steroids.

There is now ample evidence from tracer studies that adrenal steroids are derived from cholesterol, and from acetate. In the case of the latter precursor, it is not known whether adrenal steroids are synthesized directly from acetate or come from cholesterol produced from this precursor in the adrenal, the liver, or both. Experiments designed to elucidate this important problem are currently in progress.

SUMMARY

1. Tritium cholesterol is sufficiently stable to be useful for tracer studies of various aspects of cholesterol metabolism in man.

2. THE, THF, 11-ketoetiocholanolone, androsterone and etiocholanolone are derived in part from the intact tetracyclic rings of preformed plasma free cholesterol.

3. The side chain of cholesterol does not appear to be utilized for steroidogenesis.

4. In this study, about 53 per cent of the tritium was attached to the tetracyclic nucleus of cholesterol-t. The remainder was attached to the side chain.

Acknowledgments

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REFERENCES

1. WERBIN, H., and LEROY, G. V.: Cholesterol—a precursor of tetrahydrocortisone in man, *J. Am. Chem. Soc.* **76**: 5260, 1955.
2. WERBIN, H., and LEROY, G. V.: Cholesterol—a precursor of tetrahydrohydrocortisone (THF) and 11-ketoetiocholanolone in man, *Fed. Proc.* **14**: 303, 1955.
3. BIGGS, M., and KRITCHEVSKY, D.: Observations on the suitability of tritium-labeled

cholesterol f
430, 1952.
4. FUKUSHIMA.
platinum-cat
5. BLOCH, K.,
Chem. **149**:
6. FUKUSHIMA.
exchange of
7. BLECHER, M
lesterol by s
8. BLOCH, K.,
formation, *J*
9. JONES, E. R
phytosterols
10. GOULD, R.
BERGENSTAL
in man, *J. L*
11. BAGGETT, B.
urinary corti
12. BURTON, R.
steroids. II.
13. SAVARD, K.:
Chem. **202**:
14. CHEN, G.; W
cortical ster
15. CALLOW, N.
of substanc
androgen co
16. WILSON, H.
colorimetric
17. HAYES, F.
water and o
18. HECHTER,
of cholester
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19. SABA, N., a
Fed. Proc. 1
20. REICH, E.,
adrenal hon
21. NELSON, D
from the a
22. ROMANOFF,
corticostero
1546, 1953.
23. DORFMAN,
Minn., Bur
24. UNGAR, F.
vivo, *J. Bi*

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cholesterol for the study of cholesterol metabolism, *Arch. Biochem. & Biophys.* **36**: 430, 1952.

4. Fukushima, D. K., and Gallagher, T. F.: Isotopic distribution in cholesterol after platinum-catalyzed hydrogen-deuterium exchange, *J. Biol. Chem.* **198**: 861, 1952.
5. Bloch, K., and Rittenberg, D.: The preparation of deuterio cholesterol, *J. Biol. Chem.* **149**: 505, 1943.
6. Fukushima, D. K., and Gallagher, T. F.: Studies on the platinum-catalyzed exchange of hydrogen isotopes with steroids, *J. Biol. Chem.* **198**: 871, 1952.
7. Blecher, M., and Gurin, D.: The conversion of radioactive acetoacetate to cholesterol by surviving rat liver slices, *J. Biol. Chem.* **209**: 953, 1954.
8. Bloch, K., and Rittenberg, D.: On the utilization of acetic acid for cholesterol formation, *J. Biol. Chem.* **145**: 625, 1942.
9. Jones, E. R. H.; Wilkinson, P. A., and Kerlogue, R. H.: The oxidation of phytosterols with the Oppenauer reagent, *J. Chem. Soc.* p. 391, 1942.
10. Gould, R. G.; LeRoy, G. V.; Okita, G. T.; Kabara, J. J.; Keegan, P., and Bergenstal, D. M.: The use of C^{14}-labeled acetate to study cholesterol metabolism in man, *J. Lab. & Clin. Med.* **46**: 372, 1955.
11. Baggett, B.; Kinsella, R. A., Jr., and Doisy, E. A.: Hydrolysis of conjugates of urinary corticoids with β-glucuronidase, *J. Biol. Chem.* **203**: 1013, 1953.
12. Burton, R. B.; Zaffaroni, A., and Keutmann, E. H.: Paper chromatography of steroids. II. Corticosteroids and related compounds, *J. Biol. Chem.* **188**: 763, 1951.
13. Savard, K.: Paper partition chromatography of C_{19}- and C_{21}-ketosteroids, *J. Biol. Chem.* **202**: 457, 1953.
14. Chen, G.: Wheeler, J., and Tewell, H. E., Jr.: Methods of estimation of adrenal cortical steroids with tetrazolium salts, *J. Lab. & Clin. Med.* **42**: 749, 1953.
15. Callow, N. H.; Callow, R. K., and Emmens, C. W.: Colorimetric determination of substances containing grouping—$CH_2 \cdot CO$—in urine extracts an indication of androgen content, *Biochem. J.* **32**: 1312, 1938.
16. Wilson, H., and Carter, P.: Stabilization of the alcoholic potassium hydroxide in colorimetric 17-ketosteroid determinations, *Endocrinology* **41**: 417, 1947.
17. Hayes, F. N., and Gould, R. G.: Liquid scintillation counting of tritium-labeled water and organic compounds, *Science* **117**: 480, 1953.
18. Hechter, O.; Solomon, M. M.; Zaffaroni, A., and Pincus, G.: Transformation of cholesterol and acetate to adrenal cortical hormones, *Arch. Biochem. & Biophys.* **46**: 201, 1953.
19. Saba, N., and Hechter, O.: Cholesterol-4-C^{14} metabolism in adrenal homogenates, *Fed. Proc.* **14**: 775, 1955.
20. Reich, E., and Lehninger, A. L.: Conversion of cholesterol to corticosteroids in adrenal homogenates, *Biochim. & biophys. acta* **17**: 136, 1955.
21. Nelson, D. H.; Reich, H., and Samuels, L. T.: Isolation of a steroid hormone from the adrenal-vein blood of dogs, *Science* **111**: 578, 1950.
22. Romanoff, E. B.; Hudson, P., and Pincus, G.: Isolation of hydrocortisone and corticosterone from human adrenal vein blood, *J. Clin. Endocrinol. & Metab.* **13**: 1546, 1953.
23. Dorfman, R. I., and Ungar, F.: Metabolism of Steroid Hormones. Minneapolis, Minn., Burgess Publishing Co., 1953, p. 147.
24. Ungar, F., and Dorfman, R. I.: Incorporation of C^{14} in the urinary steroids in vivo, *J. Biol. Chem.* **205**: 125, 1953.

00131503.009

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