chlorotyrosines. Under strong oxidative conditions both may be formed together with chlorobromotyrosine and further oxidation products, which migrate relatively slowly on ionophoresis at pH 1.85.

This loss of tyrosine could be avoided in our experiments by the addition of small amounts of compounds that are readily oxidized, *e.g.* thioglycollic acid, or halogenated, *e.g.* phenol or hydrazine (Fig. 2). These additives may prove generally useful in preserving tyrosine during acid hydrolysis.

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SC 2228

Loss of the 4-β hydrogen in the conversion of [4-β-^{3}H] cholesterol to cortisol by the guinea-pig adrenal gland homogenate

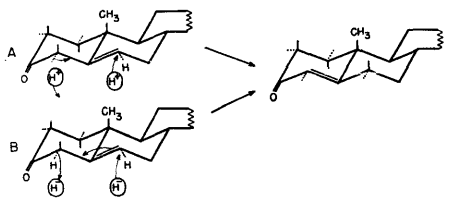
The conversion of pregnenolone to progesterone, considered one of the principal reactions in the biosynthesis of cortisol from cholesterol, has been shown to occur in perfused beef-adrenal glands[1] and in adrenal-gland slices[2]. It has also been effected by two bacterial enzymes acting consecutively: an NAD-linked 3β-ol-Δ^5-steroid dehydrogenase which oxidizes the 3β-hydroxy to a 3-keto group[3], and a 3-ketosteroid Δ^5-Δ^4 isomerase (steroid Δ-isomerase, EC 5.3.3.1) which shifts the double bond from the 5–6 to the 4–5 position, a process that involves an intramolecular hydrogen transfer from C-4 to C-6 (see ref. 4).

With the aid of cholesterol labeled with tritium almost exclusively in the β-position of C-4, by the procedure described by IRELAND *et al.*[5], we have studied certain aspects of the mechanism of Δ^5-3-ketosteroid isomerization in the intact guinea pig. Animals that had been fed a mixture of [4-^{14}C]cholesterol- and [4-β-^{3}H]-cholesterol for 10 days excreted [4-^{14}C]cortisol and 6β-[4-^{14}C]hydroxycortisol containing negligible amounts of ^{3}H. Since guinea-pig urine is alkaline, and the ethyl acetate used to extract the steroids was washed with 0.1 N NaOH, the possibility

existed that, if tritium had been present on C-4 or C-6 of these two steroids, it was lost during their isolation. Because of this possibility, we studied the conversion of a mixture of the two labeled cholesterols to cortisol by a guinea-pig adrenal homogenate[6,7] in a neutral buffer. Cortisol was isolated under neutral conditions. The results shown in Table I are in harmony with those recorded above with the intact guinea-pigs. The added cholesterol with a disintegration/min ratio of ^{3}H to ^{14}C of 1.97 was converted to cortisol with a ratio of 0.04 (Column 4). The constancy in the values for the ^{14}C-specific activities of the isolated cortisol (Column 3) is strong evidence for its radiochemical homogeneity.

The residue from the methanol eluates of blank paper used for the chromatography displaced the tritium pulse spectrum giving rise to a higher count rate in the upper window of the liquid scintillation spectrometer. Because of the losses incurred in the purification of the cortisol, less paper was involved in the elution of the cortisol in the later steps, and this probably accounts for the declining tritium values shown in Column 2. It should be noted that the labeled cortisol acetate was practically devoid of spurious tritium counts after its crystallization.

Although the conversion of cholesterol to cortisol is brought about by a number of enzymes acting at different points on the sterol molecule, it is logical to assume, in keeping with current concepts of cortisol biosynthesis, that steroid Δ-isomerase, which is concerned with altering the atomic configuration of pregn-5-ene-3,20-dione at Positions 4, 5 and 6, is responsible for the loss of the 4-β hydrogen. Tentatively then, this enzyme, in the adrenal gland, may be envisaged as effecting either of the following atomic and electronic sequences:



A mechanism somewhat analogous to that shown in sequence A has been postulated for the enzymic isomerization of isopentenyl pyrophosphate to dimethylallyl pyrophosphate[13]. The mechanism of this double bond shift has been elaborated by AGRANOFF *et al.*[14]. Sequence B involves the addition of a hydride ion to C-6 and loss of a hydride ion at C-4. The possible enzymic reduction of the carbon double bond of α-β-unsaturated ketones by hydride-ion transfer is discussed by NORCROSS *et al.*[15] and by CORNFORTH[13].

Other explanations for the findings shown in Table I may be suggested. For example, chemical enolization could have resulted in loss of tritium from position 4 of pregnenolone or from the 4 or 6 positions of cortisol. But chemical enolization of a steroid generally requires an acid or alkaline medium. In this study the incubation and the isolation of the cortisol were carried out at pH 7.1. The labilization of tritium from pregnenolone or cortisol could, of course, have been brought about by an enzymic

TABLE I

CONVERSION OF [4-^{14}C,4-β-^{3}H]CHOLESTEROL $^{3}H/^{14}C = 1.97$)* TO [4-^{14}C]CORTISOL BY GUINEA-PIG ADRENAL HOMOGENATE

4.2 g of whole adrenal glands were homogenized with 8.4 ml of Bucher buffer[8] containing 0.005 M fumarate. After centrifugation at 700 × *g* for 6 min, the supernatant was mixed with 1.65 μC of [4-β-^{3}H]cholesterol and 0.84 μC of [4-^{14}C]cholesterol, both dissolved in 0.1 ml of propylene glycol. After 2 h of incubation at 37° under oxygen, the initial pH of the homogenate 7.1 was unchanged. A boiled liver homogenate incubated with the labeled cholesterols under the same conditions served as a control. Carrier cortisol (3.0 mg) was added to each flask at the end of the incubation. Both mixtures were diluted with water and were extracted 3 times with ethyl acetate. The combined extracts were washed with water, dried over anhydrous sodium sulfate, and distilled to dryness *in vacuo*. The residue was taken up in a solution consisting of 70% methanol and 30% water. The aqueous methanol was then extracted 3 times with equal volumes of petroleum ether. The aqueous methanol was then distilled to dryness under vacuum, and the residue was chromatographed on methanol-washed Whatman No. 1 paper. Samples were dissolved in 2.0 ml of dioxane and 15.0 ml of toluene containing 45 mg of 2,5-diphenyloxazole, and radioactive measurements were made on a Packard liquid scintillation spectrometer (Model 314E). ^{3}H and ^{14}C disintegrations/min were calculated by the discriminator-ratio method[9].

Purification step	*Cortisol isolated from:*				
	Adrenal homogenate		*Liver homogenate*	*Counting solvent blank*	
	^{3}H disintegrations /min/mg	*^{14}C disintegrations /min/mg*	*^{3}H disintegrations/min / ^{14}C disintegrations/min*	*Counts/min channel 1** / counts/min channel 2*	*Counts/min channel 1** / counts/min channel 2*
1. 12-h chromatography in system A***. Cortisol eluted and counted			0.26	47 18	36 12
2. 17-h chromatography in system B*** to remove scintillator. Cortisol eluted and acetylated					
3. 12-h chromatography in system C***. Cortisol acetate (1.7 mg) eluted from paper and counted	1061	313	0.34	61 13	38 15
4. 17-h chromatography in system B***. Cortisol acetate (1.52 mg) eluted and counted	603	2940	0.21		
5. 12-h chromatography in system C***. Cortisol acetate (1.20 mg) eluted and added to 9.23 mg of non-labeled cortisol acetate. Crystallized from dioxane–toluene[10], m.p. 211.5°–215°. Counted 5.0 mg	118	3080	0.04		

* The determination of this ratio was carried out on the labeled cholesterol dibromides that had been recrystallized twice from an ether–acetic acid mixture.

** Channel 1 and 2 refer to the counts recorded for the upper and lower discriminator settings.

*** System A: benzene – methanol – water[11] (50:25:25). System B: toluene–propylene glycol[12]. System C: benzene – petroleum ether – methanol – water[11] (1.66:3.33:4,1).

reaction as yet unknown. The mechanism presented here, however, is in line with the current concept for the biogenesis of cortisol.

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Note added in proof. We recently found that acetone-washed beef adrenal mitochondria convert [4-^{14}C, 4-β-^{3}H]cholesterol to pregnenolone with tritium retention and to progesterone with loss of tritium. These findings support the view that steroid Δ-isomerase mediates the tritium loss from pregn-5-ene-3,20-dione.

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Gas-chromatographic identification of progesterone in human pregnancy plasma

Methods for the microquantitative detection of steroids have improved greatly in recent years. The association of thin-layer adsorption and gas–liquid chromatography offers the advantages of rapidity, simplicity, sensitivity as well as specificity. In the present report evidence is indicated that progesterone can be qualitatively detected in peripheral plasma by gas-chromatographic techniques.

A Research Specialties gas chromatograph (model-600) equipped with a hydrogen flame detector was used in association with a 1-mV recorder. Three columns were packed and prepared according to the methods of HORNING, MOSCATELLI AND SWEELEY[1]. The use of a solids injector device[2] allowed for the introduction of 1–30-μl aliquots of sample without significant solvent interference. The latter was kept against the "flash heater" (330°) for 30 sec.