mean values at the stages 8–9, 10–11 or 12–13 weeks of gestation ($p<0.001$) and significantly higher than the values at 14–15 weeks of gestation ($p<0.01$–0.02). The ^{3}H-NA uptake values for the mouse atria and ventricles in NA-concentration 10^{-7} M were 186 ± 21 ng/g and 143 ± 15 ng/g (mean ± S. E. M. of 10 determinations). The highest mean values, 42 and 44 ng/g found in the human foetal heart, were only about one-quarter to one-third of those found in the mouse atria and ventricles.

Histochemistry. In the mouse atria (figure 2a), quite a thick net of adrenergic terminals was seen in contrast to the human foetal heart preparations (figures 2b–e). In some of the foetal atria at 12–13 weeks of gestation, a few terminals were found around the coronary vessels and in the hearts of the foetuses at 14–15 weeks of gestation and thereafter a few fluorescent adrenergic terminals could be seen in the myocardial tissue.

Metabolites. It was found (table) that the ^{3}H activity taken up into the mouse heart was non-metabolized ^{3}H-NA in a higher proportion than that in the foetal heart, in which the quantity of deaminated metabolites proved to be rather high, especially in the older foetuses.

^{3}H-NA and its metabolites in human foetal atria and mouse atria in vitro

	Foetal heart at 8–13 weeks	Foetal heart at 14–20 weeks	Mouse heart
NA	82.4±2.7	81.5±2.6	97.1±0.5
NMN	4.8±1.2	2.9±0.9	0.5±0.2
DHPG-DHMA	6.1±1.2	11.9±1.8	2.2±0.4
MHPG-VMA	3.8±0.7	2.1±0.3	0.2±0.0
F	3.0±0.6	1.6±1.0	0.1±0.0

NA = noradrenaline, NMN = normetanephrine, DHPG = 3,4-dihydroxyphenylglycol, DHMA = 3,4-dihydroxymandelic acid, MHPG = 3-methoxy-4-hydroxyphenylglycol, VMA = vanillylmandelic acid, F = the ^{3}H activity spreading outside of the metabolites on the paper. Mean ± S. E. M. of 8–10 determinations. Quantity of metabolites expressed as percentage of total activity.

Discussion. The present results show that the uptake of ^{3}H-NA into the human foetal heart tissues was rather low during the first half of gestation as compared with the uptake of ^{3}H-NA into the adult mouse heart under the same experimental conditions. Some gradual increase in the uptake was found during the first half of the foetal life, and the development of neural NA uptake mechanisms was found to start at the beginning of the second trimester of pregnancy. The histochemical findings were in good agreement with this: formaldehyde-induced fluorescence terminals were seen after 12–13 weeks in the coronary arteries and after 14–15 weeks of gestation in the heart muscle. The present findings are also in good agreement with the findings of Walker[9], who observed that the atria from human foetuses of less than 13 weeks of gestation did not respond to field stimulation. There was clearly a higher percentage of non-metabolized ^{3}H-NA in the activity taken by the mouse heart than in that taken by the foetal heart. This finding suggests that the metabolic inactivation mechanisms play a greater part in the immature human foetal adrenergic nervous function than in the mature mouse adrenergic nervous function.

The present results suggest that during the first trimester of human foetal life the neural mechanisms are of lesser importance, and the functional development of adrenergic nervous mechanisms starts at the same time as the morphological development of adrenergic terminals in the heart at the beginning of the second trimester of pregnancy.

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Acidic metabolite of prednisolone[1]

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Summary. The metabolic fate of the 17β-ketol side chain of (21-^{3}H) prednisolone was studied with an enzyme preparation from male golden hamster liver. The acidic metabolite of prednisolone was identified by mass spectrometry as 11β, 17α,20ξ-trihydroxy-3-oxo-1,4-pregnadien-21-oic acid. The enzyme showed substrate specificity, depending on the nature of substituent on the steroid nucleus.

Evidence has been presented to support the existence of acidic corticosteroid metabolites called 'polar compounds' or 'bicarbonate extractable materials' in human urine[2,3]. 2 classes of acidic cortisol metabolites characterized by having a glycolic or glyoxylic acid side chain in human urine have been identified; they have been shown to constitute 5–25% of the administered cortisol radioactivity[4]. The formation of uncharacterized acidic metabolites of progesterone and deoxycorticosterone by the mitochondrial fraction of rabbit liver was recently reported[5]. We have presented the purification of an enzyme from hamster liver which is responsible for the oxidation of corticosteroid to steroidal-20-ol-21-oic acid[6]. In this communication the isolation and characterization of an acidic metabolite of prednisolone are described.

Steroids were bought from Research Plus Laboratory, Inc., Denville, N. J. NaB^3H_4 was purchased from New England Nuclear Corp., Boston, Mass. 21-Tritiated prednisolone was synthesized from 21-dehydroprednisolone

1 This investigation was supported in part by USPHS Grant CA 2515.
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by reduction of NaB^3H_4 as described by Monder[7]. Specific radioactivity of (21-^{3}H)-prednisolone was 36 μCi/mole. The enzyme was prepared from the cytosol fraction of hamster liver by centrifugation, ammonium sulfate fractionation, sephadex G-100 gel filtration and CM-sephadex C-50 column chromatography as described by Lee et al.[6].

Reaction mixtures were prepared to contain 0.03 μmole, total radioactivity of 1 μCi, of (21-^{3}H)-prednisolone and 1 mg of enzyme in 0.025 M tris-HCl buffer, pH 8.0, in a total 2 ml of reaction volume. Reactions were carried out at 37 °C for 6 h and stopped by freezing the mixture at −70 °C. The condensate was obtained by lyophilization of frozen incubation mixture. The remaining residue was dissolved in 2 ml of 1 N HCl solution and extracted with 5 ml of ethyl acetate twice. The distribution of radioactivity in various fractions obtained from incubation mixture is shown in table 1. The radioactivity of the condensate and ethyl acetate extract, containing both substrate and metabolite, were 9.8 and 67.8% of substrate added, respectively. The recovery of overall radioactivity averaged 79.2%.

Table 1. Distribution of radioactivity in fractionated incubation mixture

Fractions*	Radioactivity DPH × 10^{-3}	%
Condensate	240.2	9.8
Ethyl acetate extract 1	1655.7	67.8
Ethyl acetate extract 2	7.3	0.3
Aqueous phase	31.7	1.3
Total	1934.9	79.2

*The condensate was obtained by lyophilization of the reaction mixture containing radioactivity of 1 μCi and the resulting residue dissolved in 2 ml of 1 N HCl was extracted with ethyl acetate twice.

Table 2. Substrate specificity of the enzyme

Substrates	Detritiation* I DPM	%	II DPM	%
Prednisolone	1195	2.04	4327	3.60
6 -fluoroprednisolone	668	1.62	2032	2.46
6 -methylprednisolone	1493	2.24	3386	2.46
Cortisol	1403	1.04	2682	1.18
11-deoxycorticosterone	8528	17.77	17575	18.31

*Incubation mixture was prepared to contain 0.74 mg of protein and 0.5×10^{-3} μmole (I) and 1.0×10^{-3} μmole (II) of (21-^{3}H)-corticosteroids with varying specific activities.

The initial detritiation velocity was proportional to the amount of enzyme added. The rate fell off during prolonged incubation. The enzyme solution did not show any detritiation activity after being boiled for 3 min.

The accumulated ethyl acetate extracts were dried under reduced pressure and the resulting residue dissolved in methanol was applied onto silica gel plates made of silica gel G.F. 254. After the plates were developed with chloroform:methanol (98:2), 2 bands were observed under UV-light. One, at R_f 0.4, corresponded to the substrate itself; the other remained at the origin. 2 radioactive peaks coinciding with the UV opaque components were detected on the plate with a radiochromatogram scanner. The TLC behavior of the metabolite in a non-polar solvent system suggested that it was more polar than the substrate. The portion containing non-mobile metabolite was scraped off the plate and eluted with methanol, and further purified with TLC, using a polar acidic solvent system (upper phase of toluence:acetic acid:water = 50:50:10). The main UV absorbing band with R_f value of 0.35 on the plates was extracted with methanol. The isolated acidic metabolite was esterified with diazomethane and subjected to crystallization with acetone-ether system.

High and low resolution mass spectra of the acidic metabolite methyl ester obtained using direct inlet system on the AEI MS 30 were identical to those of authentic methyl 11 β, 17 α, 20 ζ-trihydroxy-3-oxo-1, 4-pregnadien-21-oate ester. The fragmentation pattern derived from these spectra revealed a relatively intense molecular ion at M^+ 392.0194, $C_{22}H_{30}O_6$, and were characteristic of a steroid nucleus of Δ^4-3-ketone with a 17 glycolate methyl ester[8].

The relative detritiation rates of a number of (21-^{3}H) corticosteroids achieved in 2 h incubation are listed in table 2. The initial detritiation rate of glucocorticoids tested was significantly lower than 11-deoxycorticosterone, indicating that oxidation of tritium at C-21 of corticosteroids was affected by the nature of the ring substituents.

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The effect of antifibrinolytic agents on wound healing in vitro

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Summary. The effect of antifibrinolytic agents (antiplasminogen activators) on wound healing was studied in vitro. All these substances caused the proliferating epithelium to change direction and migrate to stratum corneum, resulting in a everted epiboly formation.

The mechanism by which epithelial cells are directed, migrate and establish contact with the connective tissue in wound healing is incompletely known. Fibrin has been proposed to have a guiding role in this process[1] and suggested as an adhesive agent to be used in reconstructive surgery[2]. The interaction between proliferating epithelial cells and fibrin is unknown, but a fibrinolytic activity by migrating epithelial cells has been proposed[3] and epithelial cells from the buccal mucosa of man have been shown to release plasminogen activator, especially during earlier stages of cell maturation[4].

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