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THE METABOLISM OF CORTISOL AND PROGESTERONE BY CULTURED UTERINE FIBROBLASTS, STRAIN U12-705*,**

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Steroid metabolism of the liver has been studied extensively since ZONDEC[1] first observed the liver to have the capacity of inactivating steroid hormones. It has been only recently, however, that evidence pertaining to the metabolism of steroids by extrahepatic tissues have appeared in the literature[2,3]. In this report data are presented which demonstrate that human fibroblasts propagated *in vitro* convert cortisol and progesterone to a wide variety of steroid products.

METHODS

Stock cultures of human fibroblasts, strain U12-705, were propagated in medium 705 (5% chick embryo extract, 20% normal horse serum, 75% solution 703[4]) as described by SWIM AND PARKER[5]. Experiments were conducted in 16 × 150 mm tubes containing 50,000–120,000 cells in 6 ml of medium supplemented with ^{14}C-steroid (8,000–10,000 counts/min, specific activity cortisol-4-^{14}C $3.2 \cdot 10^6$ counts/min/mg, progesterone-4-^{14}C, $1.07 \cdot 10^7$ counts/min/mg). The tubes were inclined at

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an angle of 5° and incubated overnight at 37° C in a stationary position and then placed in a roller apparatus at 37° C for an additional 4 days. In several experiments solution 1066[6] was substituted for the 703 in medium 705; also cells which had been propagated for 5 days in the absence of steroid were incubated for an additional 5 days in solution 1066 containing the ^{14}C-steroid.

After incubation (tubes initially containing 120,000 cells/tube increase in the order of 6–8 fold; 5-day cultures incubated an additional 5 days in 1066 do not increase more than 2 fold) the cells and cell products were extracted four times with equal volumes of warm acetone and filtered. The acetone was removed from the filtrate by vacuum distillation in the presence of a stream of nitrogen. A chloroform extract of the aqueous residue was evaporated, redissolved in approximately 0.3 ml of 1 : 1 chloroform–methanol and chromatographed according to the hexane–formamide method of ZAFFARONI[7]. The quantitation and identification of the steroids were based on methods described previously[8,9].

RESULTS

Initial chromatography (hexane–formamide) of the extract from the fibroblasts incubated with progesterone gave a chromatogram exhibiting six radioactive peaks, Fig. 1. Each peak was rechromatographed in the appropriate system.

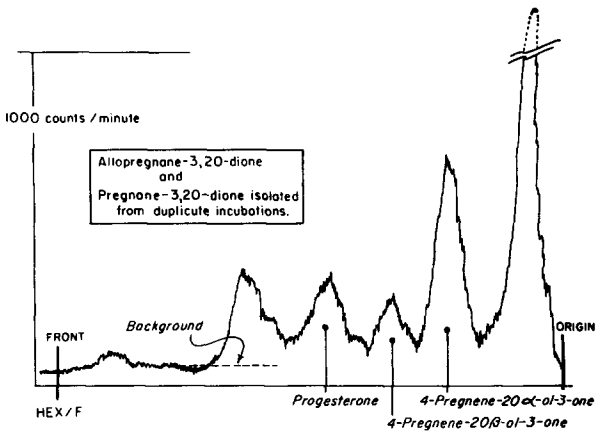

Fig. 1.

Peak I (unidentified)

Rechromatography (hexane–formamide) for 3 h gave three peaks: A (R_F 0.0), B (R_F 0.13) and C (R_F 0.23). Peak (A) is still under investigation. Peak B, upon rechromatography (benzene–formamide) separated into two peaks. B_1 migrated at the same rate as corticosterone; B_2 ran with the solvent front. Upon acetylation and rechromatography B_1 separated from the corticosterone (monoacetate) and ran with the solvent front, demonstrating that this isolated compound contained two acetylatable hydroxyl groups and was thus not corticosterone.

Peak II (4-pregnene-20-α-ol-3-one)

Rechromatography (Hexane–formamide) for 3 h with 100 μg of added 4-pregnene-20-α-ol-3-one demonstrated that the radioactive compound migrated with the carrier (R_F 0.18). The steroid in the area was eluted and divided into two portions. One portion was oxidized with CrO_3; the other was acetylated. The oxidized derivative of the mixture proved to be progesterone. The acetylated compounds migrated at the same rate on a 3-h hexane–formamide chromatogram, which confirmed the identity of 4-pregnene-20-α-ol-3-one.

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Peak III (4-pregnene-20-β-ol-3-one)

Rechromatography (hexane–formamide) of peak III demonstrated a single peak. The radioactive area was eluted and divided into two portions. One portion was acetylated in the presence of non-radioactive 4-pregnene-20-β-ol-3-one; the other was oxidized in the presence of the same compound. Both mixtures were chromatographed in the hexane–formamide system. The acetylated mixture resolved into two peaks. One ran with the acetylated carrier; the other, between the carrier and the front, demonstrated that one of the compounds of peak III was 4-pregnene-20-β-ol-3-one. The oxidized sample upon chromatography, was resolved into three peaks. The major peak corresponded with the carrier and was identified as progesterone. The other two peaks (less polar) exhibited R_F values which corresponded to allopregnane-3,20-dione and pregnane-3,20-dione. Elution of the two areas and chromatography (hexane–formamide) with the respective carriers demonstrated that the radioactive compounds migrate at the same rate as the index compounds.

In addition to the identification of 4-pregnene-20-β-ol-3-one, the above data (acetylation and oxidation) obtained for peak III gives information pertaining to the general structure of the compounds which are oxidized to allopregnane-3,20-dione and pregnane-3,20 dione. The oxidized compounds represent two or more of the pregnanolone series; *allo-* or *normal-* pregnane-20-α-ol-3-one and/or their *epi* isomers; or *allo-* or *normal-* pregnane-3-α-ol-20-one and/or their *epi* isomers.

Peak IV

This peak corresponds to unchanged progesterone.

Peak V (allopregnane-3,20-dione, pregnane-3,20-dione?)

Allopregnane-3,20-dione was added as a carrier to the eluted steroids of peak V and rechromatographed in hexane–formamide. In this chromatogram peak V was resolved into two separate peaks. The more polar peak corresponded to the carrier, allopregnane-3,20-dione. Rechromatography of the other peak in the presence of pregnane-3,20-dione gave indefinite results, but the R_F of the suspected peaks in both the original and second chromatograms were identical with that of pregnane-3,20-dione.

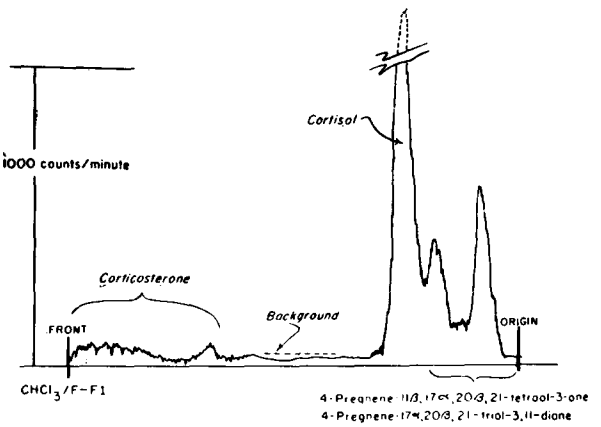

Fig. 2.

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Initial chromatography (chloroform–formamide) of the extract obtained from the fibroblasts incubated with cortisol gave a chromatogram exhibiting five peaks, Fig. 2. The three most polar peaks were combined and chromatographed (chloroform–formamide) for 6 h, which resolved the peaks into three distinct areas.

Peak I (Unidentified)

This peak, which remained at the origin, has not been further studied.

Peak II (4-pregnene-11β,17α,20β,21-tetrol-3-one) Reichstein's substance "E"

This peak, when eluted and rechromatographed, migrated at the same rate (R_F 0.14) as unlabeled Reichstein's substance "E", added as a carrier. Acetylation and chromatography (benzene–formamide) demonstrated that the radioactive compound (diacetate derivative) migrated at the same rate (R_F 0.19) as the acetylated carrier. Oxidation of the diacetate with CrO_3 and rechromatography demonstrated a diacetate derivative running identically with Reichstein's "U" diacetate.

Peak III (Unchanged cortisol and 4-pregnene-17α,20β,21-triol-3,11-dione) Reichstein's substance "U"

Acetylation and chromatography of this peak which was anticipated to be unchanged cortisol, proved to be a mixture of cortisol (identified by acetylation and oxidation) and a second component migrating at an R_F of 0.48. Rechromatography of the latter compound in the same system with non-radioactive Reichstein's "U" diacetate demonstrated both compounds to migrate at the same rate.

Peaks IV and V (Corticosterone?, 4-androstene-11β-OH-3,17-dione?)

Peaks IV and V were combined and rechromatographed with non-radioactive corticosterone and 4-androstene-11β-OH-3,17-dione. Two distinct peaks were obtained which ran identically with the carrier compounds: corticosterone (R_F 0.19; 4-androstene-11β-OH-3,17-dione R_F 0.54).

The corticosterone area was acetylated and rechromatographed in the benzene–formamide system for three hours. The acetates ran identically (R_F 0.64). The identification of corticosterone in these experiments is only tentative, since insufficient material was available for oxidation. The quantity of radioactivity of the 4-androstene-11β-OH-3,17-dione was also of low order and prevented the preparation of a second derivative*.

DISCUSSION

The observations that cultured fibroblasts metabolize cortisol and progesterone raises an important physiological question as to whether a comparable pathway of steroid metabolism exists in fibroblast cells *in situ*. The U12-705 cells employed in these studies are known to have undergone certain morphological and physiological alterations, but information pertaining to steroid metabolism in respect to degree of differentiation, is not available. Before the overall relationships between the steroid

* Neither horse serum nor medium 705 has been observed to effect conversions of steroids incubated aseptically for five days.

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metabolism of cultured cells and cells *in situ* can be assessed, it will be necessary to extend both *in vitro* and *in vivo* studies. Studies are now under way to determine whether there is an alteration of steroid metabolism between cells cultured for several generations and freshly established strains of cells from the same source.

If the steroid metabolism observed in cultured fibroblasts is the same or approximates that in the *in situ* state, the question will still remain as to whether the conversions are associated with the mechanisms of hormone action or represent a process employed by the cell to rid itself of excess steroid. These data do not exclude the possibility that unchanged steroid hormones influence the activity of the cell. Further, the fibroblast represents only one species of cell. Other cell species may or may not have similar capacities to metabolize cortisol and progesterone. It is of interest, however, that in the course of studies on inflammation, fibroblasts have been observed to concentrate cortisol for a time within their cytoplasm[10]. Cortisol is known to induce morphological changes (retraction of cell processes) in freshly isolated[10] and cultured fibroblasts[11]. Also, fibroblasts are considered by many cytologists to give rise to a variety of more differentiated cells. If this latter concept is correct, the steroid conversions observed in fibroblasts may represent a basic pattern of steroid metabolism which may be passed on in varying degrees to more differentiated cells.

The foregoing data demonstrate that progesterone and cortisol are metabolized by human uterine fibroblasts propagated *in vitro*. As fibroblasts are distributed throughout the animal organism, these data suggest the possibility that a wide variety of tissues may possess the capacity to metabolize steroids. The observations of KOCHAKIAN[2] that skeletal muscle can convert androstenedione to androstanedione and those of BERLINER AND WIEST[3] that hepatectomized rats convert progesterone-4-^{14}C to a variety of steroid products support this view. Investigations in this laboratory have also demonstrated that both human and bovine tissues such as muscle, lung, skin, facia and intestinal mucosa convert cortisol-4-^{14}C and progesterone-4-^{14}C to several steroid derivatives*.

SUMMARY

1. Cultured human fibroblasts (U12-705) metabolize cortisol-4-^{14}C and progesterone-4-^{14}C to a variety of steroid products.

2. 4-Pregnene-11β-17α,20β,21-tetrol-3-one, 4-pregnene-17α,20β,21-triol-3,20,dione have been identified and corticosterone and 11β-hydroxyandrostene dione-3,17 have been tentatively identified as products of cortisol metabolism.

3. 4-Pregnene-20β-ol-3-one, 4-pregnene-20α-ol-3-one and allopregnane-3,20-dione have been identified as products of progesterone. Evidence is also presented which demonstrates the presence of two or more additional products of the pregnanolone series (pregnane or allopregnane-20α-ol-3-one and/or their *epi* isomers; or pregnane or allopregnane-3-α-ol-20-one and/or their *epi* isomers).

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* In addition to data pertaining to steroid metabolism by cultured fibroblasts, the original report included data which demonstrated that both human and bovine tissues such as muscle, lung, skin and facia convert cortisol-4-^{14}C and progesterone-4-^{14}C to a variety of derivatives.

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BACTERIAL TRANSAMINATION OF THE STEREOISOMERS OF DIAMINOPIMELIC ACID AND LYSINE

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Two of the three stereoisomers of diaminopimelic acid are known to be metabolised by bacteria. The *meso* isomer is converted to L-lysine by diaminopimelic acid decarboxylase[1], while the *meso* and L,L-isomers are interconverted by diaminopimelic acid racemase[2]. We now present evidence that in certain bacteria all three stereoisomers may participate in transamination reactions with oxoglutaric, oxaloacetic or pyruvic acids. Transamination of both isomers of lysine was also observed.

Tests for transamination were carried out by incubating the keto acid and amino acid in the presence of pyridoxal phosphate with a cell suspension of acetone-dried bacteria at pH 8.5, and examining the reaction mixtures by paper chromatography (for method, see Fig. 1). Comparisons were also made of the ability of the cells to transaminate L- or D-glutamic acid. No investigations were made as to the stereoisomeric configuration of the products of transamination, nor was the possibility of indirect transamination eliminated. No reaction occurred in the absence of bacterial cells.

The transaminase activities of various representative organisms are shown in Table I. Only *Bacillus sphaericus*, lacking diaminopimelic acid racemase[3], was suitable for testing both *meso* and L,L-isomers, since with other organisms these two isomers were rapidly interconverted by the racemase. *Escherichia coli* mutant 26-26 and *Bacillus cereus*, both of which contain no diaminopimelic acid decarboxylase[4,2], were used to eliminate any possibility that the apparent transamination of *meso*-diaminopimelic acid was due, in reality, to a preliminary decarboxylation to L-lysine, followed by transamination of lysine.

*Meso*diaminopimelic acid actively transaminated with at least one keto acid in all the organisms tested. The rates varied, both absolutely, and with respect to glutamic acid transamination. In *B. sphaericus* the rate of transamination of *meso*-diaminopimelic acid was as high as that of glutamic acid, in *Sarcina lutea* it was lower,

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