was followed by a group of at least 5 more lines. All these lines were more distinct in the homologous system. However, a number of these lines showed a reaction of identity with the heterologous antigens.

Previously it was shown (ZAMAN and CHELLAPPAH[1]) that the antigens from eggs of *Culex* could be easily distinguished from those of *Aedes* and *Armigeres*, using the gel-diffusion technique. In this study using the 4th instar larvae of the same genera a much more complex picture is obtained. Although it was possible to distinguish *Culex* larvae from *Armigeres* and *Aedes*, there was a great deal of cross-reaction. In the case of larvae, unlike the eggs, some of the cross-reaction could be due to gut contents and other extraneous antigens. It is, therefore, suggested that for comparative studies clearer and more reliable results are likely to be obtained with egg antigens as compared to larvae.

Zusammenfassung. Mit der Gel-Diffusionstechnik lassen sich die Larven von *Culex pipiens fatigans, Aedes aegypti und Armigeres subalbatus* unterscheiden. Wegen teilweise gemeinsamen Antigenen von *Armigeres subalbatus* und *Aedes aegypti* ist eine Differenzierung dieser verschiedenen Species bei Verwendung von Larven weniger eindeutig als bei Verwendung von Eiern.

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STUDIORUM PROGRESSUS

The Isolation and Biosynthesis of Highly Polar Steroids Found in Placental Tissue

In 1960 it was demonstrated by ULSTROM et al.[1] that C-6 oxygenated steroids were present in neonatal urine, while FRANZ et al.[2] produced evidence that during pregnancy the urinary concentrations of the polar steroid 6β-hydroxycortisol is elevated above the normal non-pregnant state.

Recent work in this department (DIXON and PENNINGTON[3]) has identified the presence of the 20α-hydroxy derivative of 6β-hydroxycortisol (Compound 4c), the 20β-hydroxy derivative of 6β-hydroxycortisol (Compound 4d) and the 20β-hydroxy derivative of 6β-hydroxycortisone (Compound 3) in late pregnancy urine. A further polar steroid (Compound 4b) was also recognized, but so far has not been conclusively identified.

The source of highly polar steroids identified in pregnancy urine is uncertain and it was therefore of interest to investigate to what extent the placenta might possibly contribute to their production or alternatively provide a means of concentrating and storing some of them.

Materials and methods. Tissue: Human placentas were obtained immediately following normal full-term spontaneous delivery. These were frozen to $-14°C$ in plastic bags and maintained at this temperature until extraction was carried out.

Extraction of polar steroids: 2.5 kg of placental tissue was cut up into small pieces in the frozen state and minced by means of a mechanical meat grinder ('Kenwood', Surrey, England). The mince was extracted with 2 l of freshly redistilled ethyl acetate for 4 h at 40°C. The ethyl acetate extract was decanted off and filtered through muslin in order to remove large meat particles. A further 1 l of ethyl acetate was then added to the placental residue and extraction continued for a further 2 h at 40°C.

The combined ethyl acetate extracts, which were now bright yellow in colour were evaporated under vacuum at 37°C to a final volume of 200 ml. 400 ml of *n*-heptane were added to the ethyl acetate extract and the solution extracted with 200 ml of distilled water on three occasions. In this process much of the contaminating non-polar material is left in the organic phase and the highly polar corticosteroids pass almost quantitatively into the aqueous phase (FRANZ, KATZ, and JAILER[4]). To the aqueous extract was added 20% (w/v) anhydrous sodium sulphate and the solution was extracted with 300 ml of ethyl acetate on two occasions. The ethyl acetate was washed with two 50 ml portions of a solution of 10% (w/v) sodium carbonate and 20% (w/v) anhydrous sodium sulphate in water. (Sodium carbonate was used in preference to sodium hydroxide for washing, since only very low yields of polar steroids were expected from the placental extract and VENNING[5] had presented evidence that there can be destruction of small amounts of adrenal steroids by alkali.) The washed extract was dried over anhydrous sodium sulphate and evaporated down under vacuum at 37°C. The residue was transferred to a test tube with small volumes of chloroform:methanol (1:1) and finally with pure methanol. The solvent was then evaporated off under a stream of nitrogen at 37°C.

The residue dissolved in chloroform:methanol (1:1), was transferred to Whatman 3 mm paper as a thin band 8 cm wide. After equilibration for 3 h in benzene:methanol:water 2:1:1 by vol (BUSH[6]) the chromatogram was developed for $5\frac{1}{2}$ h. On examination under UV-light at 254 mμ, four UV absorbing zones were observed; the most intense of these zones was at the origin. A 2 mm strip from the origin to the solvent front was cut out from the centre of the 8 cm strip and dipped through a 2:1 solution of 10% aqueous sodium hydroxide in 50% methanol and 0.025% blue tetrazolium in ethyl alcohol (DIXON[7]). After heating at 60°C for 10 min, four yellow fluorescing zones were observed under UV-light at 365 mμ, each corresponding to an area which had shown UV absorption. Since it was only of interest to investigate the most polar area, the zone of the origin was subjected to further investigation. No further identification procedures were carried out on the other three zones. An area extending 2 cm each side

[1] R. A. ULSTROM, E. COLLE, J. BURLEY, and R. J. GUNVILLE, J. clin. Endocrin. Metab. *20*, 1080 (1960).
[2] A. G. FRANTZ, F. H. KATZ, and J. W. JAILER, Proc. Soc. exp. Biol. Med. *105*, 41 (1960).
[3] W. R. DIXON and G. W. PENNINGTON, to be published.
[4] A. G. FRANTZ, F. H. KATZ, and J. W. JAILER, J. clin. Endocrin. Metab. *21*, 1290 (1961).
[5] E. VENNING, Recent Progr. Hormone Res. *9*, 300 (1954).
[6] I. E. BUSH and V. B. MAHESH, Biochem. J. *71*, 705 (1959).
[7] P. DIXON, personal communication (1961).

of the origin was chromatographically eluted with ethyl acetate:methanol (2:1) and the solvent evaporated off under nitrogen at 37°C.

A portion of the residue (P_1) was taken up in chloroform:methanol (1:1) and streaked across the full width of a 3 cm limb of Whatman 3 mm chromatography paper in the form of a thin band. After equilibration overnight in benzene:ethyl acetate:methanol:water 7:3:10:10 by vol (DIXON[7]) the chromatogram was developed for 12 h. Examination under UV-light revealed two intense UV absorbing zones lying 2.5 and 11.5 cm from the origin. The strip was now treated with the alkaline blue tetrazolium (BT) reagent made up as previously described. Only one fluorescent zone was observed 20.2 cm from the origin and this zone corresponded to the location of a 6β-OH-F marker which had been run on a similar strip.

Another portion of the residue (P_1) was spotted on Whatman 3 mm paper with 20β-hydroxy 6β-OH-F, 6β-OH-F and Compound 4(b) which was previously isolated but not identified in pregnancy urine, as markers. After equilibration at 30°C overnight in t-butyl alcohol:benzene:water 43:70:86 by vol (FRANTZ et al.[4]) a 30 cm run was carried out. Examination under UV-light revealed two very intense UV absorbing spots with Rf values of 0.18 and 0.48 and the sodium fluorescence reaction showed four fluorescing spots with Rf values of 0.3, 0.44, 0.69 and 0.88. Only two BT positive spots were observed before heating: one with an Rf value of 0.69 and the other with an Rf value of 0.88, which is a similar Rf value to that of 6β-OH-E when run at 30°C in t-butyl alcohol:benzene:water 43:70:86 by vol (FRANTZ et al.[4]).

The markers gave Rf values as follows: Compound 4(b) 0.3, 20β-hydroxy 6β-OH-F 0.48, and 6β-OH-F 0.69. This is shown in Figure 1.

Conclusion. The results of this investigation would appear to indicate that there are compounds present in placental tissue more polar than 6β-OH-F. One of these soda-fluorescing compounds has the same chromatographic mobility as Compound 4(b) which was previously isolated from pregnancy urine while another soda-fluorescing compound would appear to have the same mobility as the 20α-hydroxy derivative of 6β-OH-F. The intensity of fluorescence of the former compound was noted to be about twice that of the latter compound and so it is possible that the placenta is making a significant contribution of Compound 4(b) during pregnancy.

Although no other soda-fluorescing compounds more polar than 6β-OH-F were noted on chromatograms, this does not exclude the presence of very small quantities since the limit of sensitivity of the soda-fluorescence reaction with polar steroids is about 0.02–0.05 μg/cm² (BUSH[8]).

Biosynthesis of highly polar steroids by human placental tissue. Extraction of human placental tissue had indicated that corticosteroids more polar than 6β-OH-F were present. It was of interest therefore to investigate whether the placenta itself was capable of producing these compounds or alternatively whether they had been transported there from some other site and perhaps concentrated.

Previous studies in this field have shown that the placenta possesses the necessary enzymes for hydroxylation of the C-20 (MEIGS and ENGEL[9]) and C-6 positions (BERLINER and SALHANICK[10]).

In the present investigation, the capacity of the human placenta to metabolize cortisol and cortisone was studied.

Tissue preparation and incubation procedure. Frozen tissue was sliced and finely minced. Three 25 g portions of the mince were taken and suspended in 50 ml of Krebs-Ringer phosphate buffer pH 7.4 in separate flasks. Cortisol (1 mg) dissolved in 0.5 ml of absolute ethanol was added to one flask and cortisone (1 mg) to another. The third flask, to which steroids were not added, served as a control. All three flasks were incubated at 37°C with air as the gas phase for a period of 5 h with constant shaking.

Extraction of steroids. The incubation mixture was filtered through muslin and the residue shaken with 25 ml of distilled water and again filtered. Finally the filter was washed with a further 25 ml of distilled water. To the aqueous extract was added 20% (w/v) anhydrous sodium sulphate and the solution extracted with 50 ml of ethyl acetate on two occasions. The ethyl acetate extracts were pooled and washed with 20 ml of a solution of 10% (w/v) sodium carbonate and 20% (w/v) anhydrous sodium sulphate in distilled water. The extract was dried over anhydrous sodium sulphate and evaporated to dryness under vacuum at 37°C.

Chromatography. The residue was taken up in chloroform:methanol (1:1) and put as a thin band across a 3 × 40 cm strip of Whatman 3 mm paper and after equilibration in benzene:methanol:water 2:1:1 by volume (BUSH[6]) for 3 h the chromatogram was developed for 6 h. Examination under UV-light at 254 mμ revealed an intense absorbing zone on the origin which was cut out and subsequently eluted with ethyl acetate:methanol 2:1. After removal of the solvent under a stream of nitrogen at 37°C a portion of the residue was spotted on Whatman 3 mm paper with 20β-hydroxy 6β-OH-F and 6β-OH-F as markers. After equilibration overnight at 30°C in t-butyl-alcohol:benzene:water 43:70:86 by volume (FRANTZ et al.[4]), a 30 cm run was carried out. The strip was scanned under UV-light at 254 mμ and subsequently examined for soda-fluorescence. The results, which are shown in Figure 2, may be summarized as follows:

Incubation with cortisol. Two UV absorbing spots with Rf values of 0.18 and 0.48 were observed. Soda-fluorescence showed two spots with Rf values of 0.30 and 0.69.

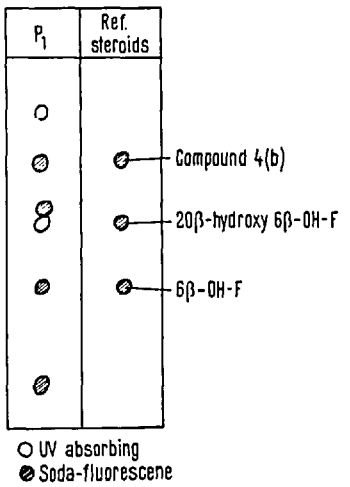

Fig. 1. Chromatography of placental extract (P_1) in t-butanol:benzene:water (43:70:86).

[8] I. E. BUSH, *Chromatography of Steroids* (Pergamon Press, London 1961).
[9] R. A. MEIGS and L. L. ENGEL, Endocrinology *69*, 152 (1961).
[10] D. L. BERLINER and H. A. SALHANICK, J. clin. Endocrin. Metab. *16*, 903 (1956).

6β-OH-F gave an Rf of 0.69. No fluorescence was observed in the region of the 20β-hydroxy 6β-OH-F marker.

Incubation with cortisone. Two UV absorbing spots with Rf values of 0.18 and 0.48 were observed. Only one soda-fluorescing spot was seen with an Rf value (0.88) comparable to 6β-OH-E when run in a similar solvent system at 30°C.

Control. Only two UV absorbing spots with Rf values of 0.18 and 0.48. No soda-fluorescence at all was observed.

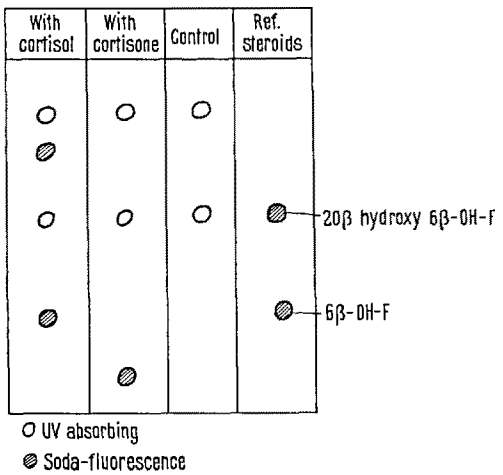

Fig. 2. Chromatography of the products after incubation of placental tissue with cortisol and cortisone.

Conclusions. On the basis of chromatographic mobility, this present work suggests that the human placenta possesses the necessary enzymes for the conversion of cortisol to a Δ^4-3-ketosteroid with a glycerol side chain more polar than the 20β-hydroxy derivative of 6β-OH-F and the 20α-hydroxy derivative of 6β-OH-F. This compound has the same chromatographic mobility as Compound 4(b) which was previously isolated from pregnancy urine. Since no soda-fluorescing material was observed on chromatograms in the region of either 20α-hydroxy 6β-OH-F or 20β-hydroxy 6β-OH-F after the incubation of cortisol with placental tissue, it would appear that the placenta is contributing to the production of the unidentified polar steroid Compound 4(b) but that the 20-hydroxy derivatives of 6β-OH-F are being produced at some other site and transported to the placenta. This latter suggestion is supported by the fact that soda-fluorescing material with the chromatographic mobility of 20α-hydroxy 6β-OH-F was only observed in extracts from large amounts of placental tissue.

Zusammenfassung. Es wird nachgewiesen, dass die menschliche Plazenta enzymatisch Cortisol in ein Δ^4-3-Ketosteroid mit Glycerol-Seitenkette, welches mehr polar ist als die 20α- und 20β-Derivate des 6-β-OH-Cortisols, umwandeln kann. Es scheint, dass die Plazenta dieses Steroid das aus dem Urin schwangerer Frauen isoliert wurde, selbst bildet.

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Coplanare Hetero-oligobasen (Phthalanilide), hochaktive Cytostatica[1]

Ausgehend von theoretischen Vorstellungen über Teilprozesse des aktiven Transports von Ionen durch die Zellmembran unter Beteiligung von Lecithin haben HIRT et al. im Forschungsinstitut Dr. Wander AG in Bern etwa 900 Stoffe einer Substanzklasse synthetisiert, in der Hoffnung, dass diese Verbindungen, als polyvalente Basen, mit Phosphatiden komplexieren, sich somit als potentielle Hemmsubstanzen des Ionentransports durch die Phosphatidfacetten der Zellmembran und damit letzten Endes als Cytostatica eines neuartigen Wirkungstypus erweisen möchten[2]. Bei der routinemässig erfolgenden Testung im primären Screeningvorgang des Cancer Chemotherapy National Service Center (CCNSC) in Bethesda (USA), erwiesen sich viele Substanzen dieser Gruppe als hochaktiv gegen die Mäuseleukämie L 1210 und einige ihrer Varianten[3], ferner gegen einige in der Ascitesform, aber nicht in der soliden Form wachsende Tumoren. Da eine grössere Anzahl dieser Stoffe auch gegen *Mycobacterium tuberculosis* in vitro cytostatisch wirksam ist, erschien die Erforschung des molekularen Wirkungsmechanismus der einzelnen Vertreter dieser Stoffklasse notwendig.

In der bis jetzt vorliegenden amerikanischen Literatur wurde diese Stoffklasse mit dem Oberbegriff «Phthalanilide» belegt. Die repräsentativen Grundtypen weisen ein lateral-symmetrisches Molekelsystem auf, in dem mindestens zwei stark basische Gruppen durch mehrere aromatische Ringe und durch Carbonamidgruppen verbunden sind, derart, dass die Molekel durchgehend planar ist. Die lateralen Adnexe können an dem Mittelring *para-* oder *meta*ständig gebunden sein, auch kann dieser noch andere Substituenten tragen. Die nächstfolgenden aromatischen Ringe können vom Mittelring durch Carbonamid-, Ureid- oder noch längere Heterogruppen getrennt sein, und schliesslich besteht auch eine grosse Variationsmöglichkeit in der Ausbildung der basischen, endständigen Gruppierungen, ob offene Anordnungen oder 5- bzw. 6-Ringe

[1] Ausgeführt mit Unterstützung durch den Krebsforschungsfonds des Kultusministeriums Nordrhein-Westfalen.

[2] R. HIRT und R. BERCHTOLD, Med. exp. *2*, 269 (1960); Exper. *17*, 418 (1961).

[3] S. A. SCHEPARTZ, I. WODINSKY und J. LEITER, Cancer Chemother. Rep. *19*, 1 (1962). – J. M. VENDITTI, A. GOLDIN und I. KLINE, Cancer Chemother. Rep. *19*, 5 (1962). – L. W. LAW, Cancer Chemother. Rep. *19*, 13 (1962). – J. H. BURCHENAL, M. S. LYMAN, J. R. PURPLE et al., Cancer Chemother. Rep. *19*, 19 (1962). – R. F. PITTILLO, L. L. BENNETT JR., W. A. SHORT et al., Cancer Chemother. Rep. *19*, 41 (1962). – W. I. ROGERS, I. M. YORK und C. J. KENSLER, Cancer Chemother. Rep. *19*, 67 (1962).