Table I. Effect of ergocornine on ovarian 20α-HSD in pseudopregnant rats

Group	Days of treatment	Day of sacrifice	No. of animals	Rats with 20α-HSD negative C.L.
1 PP I	1	2	5	5
1 PP II	1–2	3	6	5
1 PP III	1–2–3	4	5	0
1 PP IV	1–2–3–4	5	5	0
3 PP III	3–4–5	6	6	0
5 PP III	5–6–7	8	7	0
Controls	–	5	4	4
Controls	–	9	5	5

Table II. Effect of ergocornine on ovarian 20α-HSD activity in pregnant rats

Group	Days of treatment	Day of sacrifice	No. of animals	Rats with 20α-HSD negative C.L.
1 P I	1	2	6	6
1 P II	1–2	3	5	4
1 P III	1–2–3	4	6	0
6 P III	6–7–8	9	6	1
7 P III	7–8–9	10	6	3
8 P III	8–9–10	11	7	6
9 P III	9–10–11	12	6	6
10 P III	10–11–12	13	6	6

administrations. This effect can be observed in all the animals treated only when the treatment is applied within the fifth day of pregnancy. Treatment initiated on sixth, seventh or eighth day results in correspondingly fewer animals showing this activity. After the eighth day no effect on the enzyme activity can be observed.

Discussion. From the data previously shown it appears that ergocornine treatment causes the onset of 20α-HSD activity both in pseudopregnant and in pregnant C.L. In the pregnant rat this effect is always present only when ergocornine is given within the first 5 days of pregnancy and in fewer cases when given after the fifth but before the eighth day. It is known that, in the rat, implantation takes place only from the fifth day after mating and is complete by the eighth day.

In this period of gestation (first to eighth day), as in pseudopregnancy, the ovary and particularly the luteal 20α-HSD activity is mainly controlled by the pituitary while in the following stages of pregnancy the chorionic secretion plays its considerable role[8–10].

The ergocornine effect on the C.L. in the rat is thus displayed in those situations in which the C.L. itself is mainly under hypophysial control.

When confronting our histochemical results with the findings of LINDNER and SHELESNYAK[2], it is possible to point out that the production of 20α-HP depends on the onset of 20α-HSD activity in the C.L. and to confirm the suggestion that the ergocornine acts on the hypophysial regulation of the C.L.[11].

Riassunto. Trattando ratte gravide o pseudogravide con ergocornina si può osservare la comparsa dell'attività 20α-idrossisteroide-deidrogenasica nei corpi lutei rispettivamente gravidici o pseudogravidici nei quali tale attività normalmente manca. Perchè questo effetto si manifesti sono necessari 2–3 giorni di trattamento. Si constata che l'ergocornina causa la comparsa dell'attività enzimatica sopra indicata in qualsiasi periodo della pseudogravidanza, e nel periodo della gravidanza precedente l'impianto dell'uovo. L'ergocornina quindi agirebbe tramite l'ipofisi dalla cui regolazione dipende l'attività 20α-idrossisteroide-deidrogenasica dell'ovaio.

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The Duration of Action of the Synthetic Pentacosapeptide D-Serine[1]-Norleucine[4]-Valinamide[25]-β-1-25-Corticotrophin (DW-75) in Man

A synthetic analogue which has three modifications of the amino acid sequence of naturally occurring corticotrophin, has been synthesized[1]. The polypeptide, D-serine[1]-norleucine[4]-valinamide[25]-β-1-25 corticotrophin, has been named DW-75 (Sandoz). The modifications to the amino acid sequence were introduced with the expectation that they would delay inactivation and degradation of the polypeptide by carboxypeptidases and aminopeptidases.

DOEPFNER[2] found that DW-75 has an activity of 625 IU/mg when assayed by the adrenal ascorbic acid depletion test[3]. The assay value obtained with this compound is six times that obtained with synthetic porcine 1–39 corticotrophin[4] and synthetic 1–24 corticotrophin (Synacthen, CIBA)[5].

In view of the large discrepancy between the weight of DW-75 and the results obtained by biological assay, the present investigation has been undertaken comparing the duration of activity of equal weights of porcine corticotrophin and DW-75 following i.m. injection in normal healthy volunteer subjects. Further studies were carried out following the administration of equal quantities of porcine corticotrophin and DW-75 based on the biological assay. For purpose of comparison, the duration of activity of porcine corticotrophin suspended in gelatine was determined. Further studies on the duration of activity

of DW-75 following i.v. administration have been carried out at various dosage levels.

Methods. At 900 h on separate days, at weekly intervals, normal subjects (aged 26–35 years) were given an i.m. injection of 40 IU of DW-75 (equivalent to 65 μg) and 250 IU of DW-75 (equivalent to 400 μg). 5 subjects were given 40 IU of highly purified lyophilised porcine corticotrophin (Armour) equivalent to 400 μg dry weight of material. The assay values obtained with the Sayers test for DW-75 was 625 IU/mg and for porcine corticotrophin 100 IU/mg. 5 of the subjects were given i.m. injections of 40 IU of a gelatin corticotrophin preparation (acthar gel). Blood samples were taken before and at 1, 3, 5, 7, 9, 12 and 15 h after the injection of the test substance for estimation of plasma 11-hydroxycorticosteroids (plasma 11-OHCS; plasma cortisol). Urine was collected in 2 six-hour-periods following the injection of the test substances for measurement of urinary 11-hydroxycorticosteroids (urinary 11-OHCS; urinary cortisol) concentration.

Doubling amounts of DW-75 from 31.25–125 ng were given i.v. to 5 subjects with the precautions previously described[6]. Blood samples were taken before and at 10, 15, 20, 30 and 60 min after each injection. Similar tests were carried out on 9 further subjects who were given identical doses of synacthen. 7 subjects were given 250 μg DW-75 i.v. administered as a single dose. Blood was taken before and 1, 3, 5, 7, 9, 12 and 15 h after the injection, for measurement of the plasma 11-OHCS concentration. The tests were carried out under dexamethasone suppression[6].

Plasma 11-OHCS were measured by the method of MATTINGLY[7]. Urinary 11-OHCS were measured by the method of MATTINGLY et al.[8].

Results. Figure 1 shows the plasma and urinary 11-OHCS levels following the administration of porcine corticotrophin and DW-75 in high and low dosage in one subject. The plasma 11-OHCS concentrations following the administration of i.m. DW-75 in high and low dosage and porcine corticotrophin are shown in Table I. A *t*-test on means of the paired differences in plasma 11-OHCS levels of all subjects at each time after the administration of 40 IU (400 μg) lyophilised porcine corticotrophin and 40 IU (65 μg) of DW-75 showed that the porcine corticotrophin produced a greater rise of plasma 11-OHCS at 1, 3 and 5 h (1 h $P < 0.05$, 3 h $P < 0.02$, 5 h $P < 0.05$). At 7, 9, 12 and 15 h, the differences were not significant. The means of the paired differences after the administration of 40 IU (400 μg) porcine corticotrophin and 250 IU (400 μg) of DW-75 were not significantly different at all times.

The plasma 11-OHCS levels following Acthar gel are shown in Table I. The levels obtained were significantly higher at 5, 7, 9, 12 and 15 h when compared with the elevation produced by DW-75 at both dosage levels and lyophilised porcine corticotrophin.

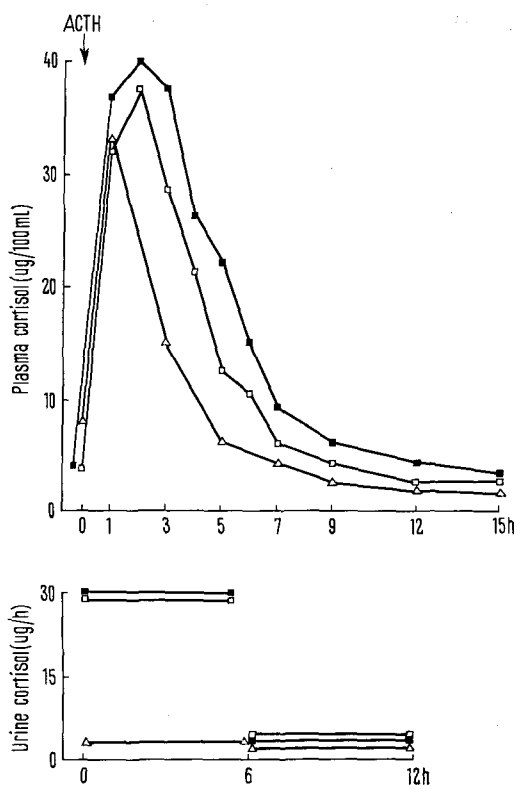

Fig. 1. The plasma and urinary 11-OHCS levels in a normal subject, at various times following DW-75 and lyophilised porcine corticotrophin. ■—■, porcine (40 IU); △—△, DW-75 (40 IU); □—□, DW-75 (225 IU).

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Table I. The plasma 11-OHCS; mean ± S.E. (μg/100 ml) following i.m. injection of DW-75 and porcine corticotrophin

Preparation	Amount (IU)	Weight (μg)	h after injection 0	1	3	5	7	9	12	15
DW-75	40	65	3.5 ± 0.8	25.0 ± 2.0	10.2 ± 1.6	5.4 ± 1.0	3.5 ± 0.5	3.2 ± 0.5	2.6 ± 0.4	2.5 ± 0.5
DW-75	250	400	2.9 ± 0.2	29.7 ± 1.0	23.2 ± 3.6	10.8 ± 2.0	5.7 ± 0.7	4.0 ± 0.2	3.1 ± 0.3	2.9 ± 0.4
Porcine corticotrophin lyophilised	40	400	3.1 ± 0.4	29.2 ± 1.9	22.0 ± 3.8	13.6 ± 2.8	7.6 ± 1.4	4.6 ± 0.5	3.0 ± 0.3	2.8 ± 0.2
Acthar gel	40	400	2.8 ± 0.4	23.0 ± 2.4	35.2 ± 3.1	38.4 ± 6.7	30.7 ± 6.3	25.2 ± 5.5	15.1 ± 4.2	8.3 ± 1.8

The Table II shows the results of the urinary 11-OHCS levels. The excretion of urinary 11-OHCS were significantly less during the first 6 h following 40 IU DW-75 when compared with 250 IU DW-75, lyophylized porcine corticotrophin and acthar gel. During the second 6 h, the urinary 11-OHCS following acthar gel were significantly elevated when compared with the levels obtained following DW-75 and lyophilised corticotrophin.

The plasma 11-OHCS concentration following 31.25, 62.5, 125 and 250,000 ng of DW-75 and Synacthen are shown in Table III. Figure 2 shows the individual values obtained in this group of subjects. The differences between the rise in plasma 11-OHCS induced by DW-75 and synacthen were not significant at all dosage levels.

Table IV shows a comparison between the levels of plasma 11-OHCS obtained following DW-75 given i.m. and i.v. The levels obtained following i.v. administration were significantly greater at 3, 5, 7, 9 and 12 h ($P < 0.01$ at all these times). At 0, 1 and 15 h the differences were not significant ($P < 0.5$).

Discussion. DW-75 is a preparation which stimulates the adrenal cortex in man when given by i.m. injection. Porcine corticotrophin produces a greater rise in plasma 11-OHCS than DW-75 when administered in equivalent doses based on the biological assay. The maximum level of plasma 11-OHCS achieved and the duration of activity of equal quantities by weight of porcine corticotrophin and DW-75 are approximately equal. When one takes into account that the molecular weight of the pentacosapeptide is just a little more than half that of the porcine corticotrophin, this discrepancy in activity becomes even more marked. It is evident that the assay values based on rat adrenal ascorbic acid depletion bear little relationship to the behaviour of synthetic polypeptides with corticotrophin-like activity when given by i.m. injection to human subjects.

JENNY et al.[9], have measured the plasma 17-hydroxycorticosteroids following the administration of DW-75 in human subjects. They found a longer duration of activity when DW-75 was given i.v. when compared with i.m. and s.c. administration. The present study would support this conclusion. Both the duration of activity and maximal level achieved are greater when administered i.v. than when given i.m. DW-75 is probably resistant to enzymatic breakdown in the blood stream but is destroyed by tissue enzymes at the site of injection.

The adrenal gland is extremely sensitive to corticotrophin stimulation. Intravenous injection of 31.25 ng of synacthen produces a rise in plasma 11-OHCS[6]. This is less than 10^{-4} of the quantity used in the usual tests of adrenocortical function which employ pharmacological

Table II. The urinary 11-OHCS; mean ± S.E. concentration expressed as μg/h, following i.m. injection of DW-75 and porcine corticotrophin

Preparation	Amount (IU)	Weight (μg)	h after injection 0–6	7–12
DW-75	40	65	5.6 ± 1.0	3.0 ± 0.4
DW-75	250	400	17.0 ± 5.4	4.7 ± 0.6
Porcine corticotrophin lyophilised	40	400	17.0 ± 5.4	4.8 ± 1.0
Acthar gel	40	400	22.2 ± 6.1	26.0 ± 5.9

Table III. The maximal plasma 11-OHCS mean ± S.E. (μg/100 ml) response to increasing doses of DW-75 and synacthen administered i.v. to normal subjects

Dose (μg)	DW-75[a]	Synacthen[b]
0	2.7 ± 0.3 (2.2–3.0)	3.0 ± 0.4 (2.4–4.0)
31.25	3.4 ± 0.4 (2.2–4.0)	5.6 ± 0.7 (3.2–10.0)
62.5	5.2 ± 0.6 (4.0–6.8)	10.2 ± 1.7 (3.4–22.0)
125	9.2 ± 0.5 (8.6–11.2)	15.3 ± 2.1 (5.6–28.0)
250,000[c]	22.3 ± 0.5 (20.8–23.6)	26.8 ± 1.9 (20.6–36.0)

[a] 5 subjects. [b] 9 subjects. [c] The values given were obtained 30 min after the administration of this dose. The figures in parenthesis represent the range of each series of observations.

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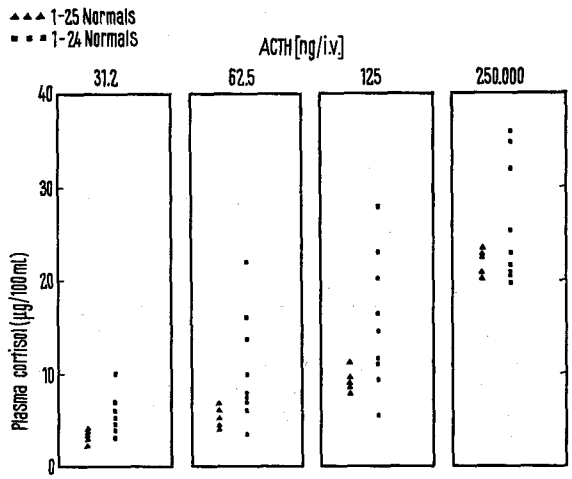

Fig. 2. The maximal level of plasma 11-OHCS following i.v. administration of increasing amounts of DW-75 (triangles) and synacthen (squares).

Table IV. The plasma 11-OHCS; mean ± S.E. (μg/100 ml) following i.v. and i.m. injection of DW-75

Route	Dose (μg)	h after injection 0	1	3	5	7	9	12	15
Intramuscular	400	2.9 ± 0.2	29.7 ± 1.0	23.2 ± 3.6	10.8 ± 2.0	5.7 ± 0.7	4.0 ± 0.2	3.1 ± 0.3	2.9 ± 0.4
Intravenous	250	2.6 ± 0.4	29.3 ± 1.4	40.2 ± 2.9	31.4 ± 2.1	18.7 ± 2.0	11.2 ± 1.0	6.8 ± 0.7	2.8 ± 0.5

quantities of corticotrophin. The administration of doses of corticotrophin which approach the minimal quantities required for stimulation of the adrenal cortex are valuable in determining the sensitivity of the gland. The procedure also has value in the biological assay of corticotrophin and its synthetic analogues, using man as the test animal. There appears to be no difference between the degree of adrenal stimulation produced by DW-75 and synacthen when given i.v. in nanogram amounts[10].

Résumé. L'activité de la corticotrophine porcine a une durée plus longue que celle du DW-75, quand on l'administre en quantités égales, basées sur un test biologique. Le DW-75 a une durée d'activité plus longue par injection intraveneuse que par injection intramusculaire. Une quantité nanagramme du DW-75 a la même action stimulante adrénocorticale que le synacthène.

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[10] I am most grateful to Dr. E. R. EVANS of Sandoz for supplies of DW-75 used in this study.

The Relationship Between Human Chorionic Somato-Mammotropine Hormone and Thyrostimuline in Biological and Radioimmunological Assays

A new protein hormone was isolated and purified in recent years from the placenta[1,2]. It was found in the blood of pregnant women and in cases of chorionic tumour[3,4]. This hormone, human chorionic somato-mammotropine (HCSM), has previously been called human placental lactogen (HPL), chorionic growth hormone, prolactine (CGP) and placental purified protein hormone (PPPH)[5].

A cross reaction between this hormone and the somatotropin STH was observed[6,7]; moreover the HCSM has a biological action similar to STH and also to prolactin action[1,2,6-8].

This placental hormone has a molecular weight between 36,000 and 38,000[9,10] and possesses some similar amino acid composition and a sequence with one part of the STH chain[10-12]. These last observations might explain the cross reaction existing between STH and HCSM and the high level of STH found both with radioimmunoassay and with biological assay during the pregnancy. This increase, however, is not due to STH but to HCSM. Indeed YEN et al.[13] have shown that STH is not increased during pregnancy; moreover, the growth hormone cannot interfere with the HCSM values, as these are about a thousand times more elevated than the STH values. A radioimmunoassay had been developed for the determination of HCSM in blood and biological fluids[3,4,14,15,21]. Since in the state of pregnancy an increased level of plasma TSH[16] was observed, it appears necessary to check also the possibility of an interference of the HCSM or of a cross reaction between HCSM and TSH in radioimmunological and biological assays. TSH could have some similar amino acid sequence with HCSM which would react with the antiserum in the radioimmunoassay, or could have some TSH-like biological activity. These problems are the purpose of this paper.

Methods. The radioimmunoassay of HCSM was described recently[4]. The radioimmunoassay for the TSH was established, as previously explained, using both bovine or human TSH antisera[17,18] and the biological assay of TSH according to MACKENZIE's method[19].

Results. The Figure shows the decreasing radioactivity of the antigen-antibody complex, as increasing quantities of unlabelled HCSM (kindly supplied by Dr. P. Neri, Isut Sclavo, Siena, Italy) are added to the incubation medium. When TSH, either bovine or human, is added, no change in the binding capacity occurs. No cross reaction is observed between HCSM and TSH in the radioimmunoassay of the HCSM. In a second experiment we have studied the possible influence of increasing quantities of HCSM on the TSH radioimmunoassay. No proportionality was observed and under a dilution of HCSM of 10 µg/ml of HCSM, no TSH was detected; but with a concentration of 100 µg/ml or more, a mean value of 0.27 mU of TSH per milligram of HCSM was detected, in the case of the radioimmunoassay using the United States Pharmacopae (USP) bovine TSH reference standard and bovine antibodies. With the human TSH standard A of National Institute of Medical Research (London) (NIMR), and human TSH antibodies a mean of 0.19 mU of TSH per milligram HCSM was found. If we suppose a mean value of 10 µg/ml of HCSM during pregnancy, it would indicate a contamination in TSH of 0.0027 or 0.0019 mU/ml of plasma for the bovine or

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