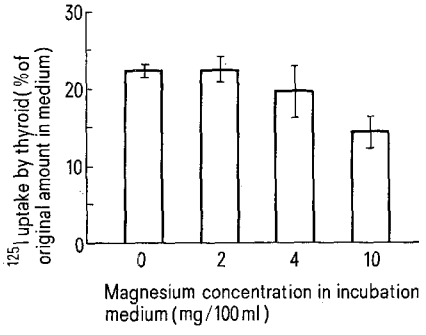
significant differences in ^{125}I accumulation by the thyroids were observed (Table). Glands from magnesium-deficient and control rats accumulated almost identical amounts of radioiodine but there was a suggestion of reduced uptake by glands from magnesium-loaded rats ($0.2 > p > 0.1$). However, this tendency, like the reduced ^{125}I uptake observed in the first experiment, is the converse of that found in vivo during magnesium loading. The smaller ^{125}I uptake by all thyroids in the second experiment than in the first may be due to the food restriction and lower growth rate of experimental than stock rats.

The results of these experiments therefore indicate that the influence of magnesium status on iodide uptake by the thyroid in living rats is not due to a direct action of either extracellular or intracellular magnesium on iodide transport by the gland and it appears likely to be secondary to other effects of the deficiency that may be occurring elsewhere in the body.



Uptake of ^{125}I by thyroid glands of stock rats incubated in media containing different concentrations of magnesium. Vertical bars indicate ± SEM, $n = 4$ for each column.

Résumé. La quantité d'^{125}I absorbée dans le médium par les glandes thyroïdes incubées in vitro ne fut pas sensiblement affectée par la variation physiologique de la concentration du magnésium dans le médium, ni par la quantité de magnésium absorbée par les rats avant leur mort.

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Placental Impermeability to Maternal ACTH in the Rabbit

The permeability of the placental barrier to several protein hormones has been studied by means of radioactive tracers. It was shown that insulin[1], HGH[2,3], TSH[4] and glucagon[5] do not cross the placental barrier. Recently, Allen et al.[6] reported the presence of high ACTH levels in mother and foetus at delivery and, from the results observed in a Nelson's syndrome and in an anencephalic foetus, they suggested that there is no significant transfer of maternal ACTH to the foetus in humans. The purpose of the present paper was to verify this lack of placental permeability to maternal ACTH by injecting labelled ACTH into pregnant rabbits.

Materials and methods. 1–39 synthetic human ACTH, kindly supplied by Ferring A. B. (Sweden), was labelled with I^{125}, according to Greenwood et al.[7]. Purification of the labelled hormones was carried out as previously described[8] and I^{125}-ACTH was used within the first 24 h after labelling.

Two pregnant rabbit females nearing term, weighing 4.3 and 5.5 kg respectively, were used for the experiment.

In the first experiment, the animal (4.3 kg) was anesthetized with Nembutal (50 mg/kg body weight). After exposing both femoral veins, one vein was injected with I^{125}-ACTH (approx. 22.10^6 cpm) diluted in 1 ml of homologous plasma. Blood samples were collected from the other vein before and 2.5, 5, 7.5, 10 and 15 min after the injection. The uterus was then removed and blood samples were immediately taken from each foetus as well as pieces of each placenta. Fragments of kidneys, liver and adrenal gland were taken from the foetus and from the mother; 0.2 ml of blood from each sample and weighed fragments of each maternal or foetal organ were dissolved in 1 ml Soluene 100 (Packard) at 37 °C for 24 h and counted in an autogamma counter.

Table I. First experiment: Radioactivity in maternal and foetal blood (cpm/0.2 ml), and organs (cpm/mg)

Maternal blood		Fetal blood	
Prior to I^{125}-ACTH injection	0	Fetus No.	
Time after I^{125}-ACTH (min)		1	60
$2^1/_2$	18.598	2	140
5	12.465	3	190
$7^1/_2$	10.590	4	217
10	9.195	5	156
15	6.983	6	133
		7	219
		8	91
		9	177
		Mean value	153
		Standard error	18
		Maternal radioactivity at 15 min = 2.20%	

Maternal organs		Fetal organs (Mean ± standard error)	
Kidney	522.7	Kidney	0.78 ± 0.25
Liver	54.5	Liver	1.25 ± 0.30
Adrenal gland	60.5	Adrenal gland	—
Ovary	45.0	Placenta	70.35 ± 8.04

In the second experiment, both femoral veins of the anesthetized animal (5.5 kg) were exposed and a catheter was inserted into each vein. One catheter was connected with a peristaltic pump, the other was used to collect blood samples. At the beginning of the experiment, 2 ml of a mixture consisting of I^{125}-ACTH (73.4%) and I^{125} (26.6% as cpm, altogether 28.10^6 cpm) was injected i.v.; 30 sec later, the i.v. infusion of the same mixture diluted with rabbit plasma was started at a rate of 1.35×10^6 cpm/0.5 ml/min.

Perfusion was maintained until the end of the experiment, i.e. for 22 min, and blood samples were collected immediately before the beginning of the experiment, 30 sec after the i.v. injection, i.e. just prior to starting the i.v. infusion, and then every 2 min for the duration of the experiment. The uterus was then removed and blood samples were rapidly collected from each foetus.

0.2 ml of each sample of maternal and foetal blood were dissolved in 1 ml Soluene 100 for 24 h at 37 °C before counting. 0.1 ml of the serum from every sample was submitted to chromatoelectrophoresis on Whatman 3 MM paper according to YALOW and Berson[9] and counted for radioactivity in a radiochromato-scanner and/or submitted to autoradiography using Ciba-Ilford films.

Results and discussion. First experiment. After injecting I^{125}-ACTH into the maternal circulation, the radioactivity in the maternal blood decreased gradually until the end of the experiment (Table I). The very low radioactivity (153 ± 18 cpm, corresponding to 2.2% of that measured in the maternal blood) found in foetal blood did not allow any qualitative study of its nature, i.e. possible degradation products of I^{125}-ACTH.

Concentration of the radioactivity was the same in the various maternal organs, except for the kidney, where higher levels were found. This observation is in agreement with the major role played by the kidneys in the catabolism of protein hormones[10]. The placenta showed a radioactivity of the same order as that found in the maternal organs (Table I).

These results suggest that the I^{125}-ACTH injected into the maternal circulation does not cross the placental barrier. However, the very low radioactivity found in the foetal circulation requires further investigation.

Second experiment. In the maternal circulation, the radioactivity (Table II) showed a prompt increase after injecting a mixture of I^{125}-ACTH and free I^{125}. It rapidly decreased and remained constant during the whole period of the i.v. infusion. At the end of the experiment, the

Table II. Second experiment: Radioactivity in maternal and foetal blood (cpm/0.2 ml)

Before i.v. injection	0	Fetus No.	
30 sec after, just prior		1	2.465
to starting i.v. infusion	22.690	2	2.695
Time after infusion (min)		3	3.201
2	14.998	4	2.364
4	14.900	5	3.173
6	13.906	6	3.140
8	14.167	7	2.905
10	13.965	8	3.796
12	14.011	9	4.098
14	14.457	10	2.730
16	14.349	11	2.938
18	14.831		
20	14.838	Mean value	3.045
22	14.430	Standard error	158
		Maternal radioactivity at 22 min = 21.1%	

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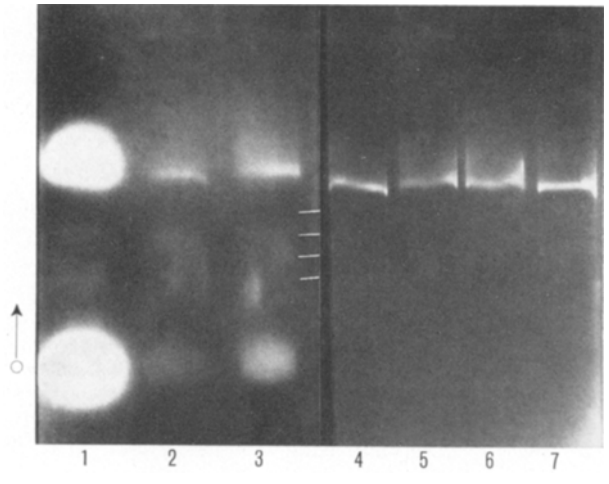
Table III. Second experiment: Serum radiochromatoelectrophoresis: % of total paper radioactivity in the various zones

Materials	Origin	Plasma proteins zone	Free I^{125} zone
Infused solution	68.2	5.2	26.6
Plasma 30 sec after i.v. injection	56.4	16.3	29.3
Plasma after starting infusion (min)			
2	52.3	29.3	18.4
4	51.7	30.4	17.9
6	48.3	30.9	20.8
8	46.2	34.8	19.0
10	45.4	35.4	19.2
12	44.6	36.7	18.7
14	43.9	37.1	19.0
16	44.1	36.8	19.1
18	43.8	37.6	18.6
20	43.6	37.6	18.8
22	43.3	37.8	18.9

radioactivity concentration in foetal blood amounted to 21.1% of that found in the mother.

As shown in Table III, chromatoelectrophoresis of the maternal serum samples and of the infused solution showed a progressive decrease of the radioactivity at the origin corresponding to the undamaged hormone, from 56.4 to 43.3%, in the maternal circulation. Simultaneously, radioactivity in the plasma protein zone (degraded hormone) increased from 16.3 to 37.8%. Free I^{125} decreased from 29.3 to 18.9%.



Second experiment: Chromatoelectrophoresis and autoradiography: (from the left) 1st paper strip: I^{125}-ACTH and I^{125} mixture used for i.v. infusion. 2nd strip: maternal serum 30 sec after the i.v. injection and prior to infusion. 3rd strip: maternal serum after 22 min of i.v. infusion. 4th to 7th strip: foetal serum. Note the presence of free I^{125} alone.

The low radioactivity of the foetal serum required autoradiography of the chromatoelectrophoretograms. As shown in the Figure, only free I^{125} was found in the foetal circulation. The results of this study indicate that, in rabbits, I^{125}-ACTH does not cross the placental barrier and they confirm foetal autonomy of its ACTH secretion.

Résumé. Les auteurs, utilisant de l'ACTH-I^{125}, ont étudié le problème de la perméabilité placentaire à cette hormone chez la lapine. Les résultats obtenus démontrent que l'ACTH maternelle ne passe pas au-delà de la barrière placentaire et confirment l'indépendance foetale des concentrations plasmatiques d'ACTH.

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21 November 1974.

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Moulting Hormone in *Locusta migratoria*: Rate of Ecdysone 20-Hydroxylation and Excretion During the Last Larval Instar

The moulting hormone of insects appears to occur in two different forms: ecdysone (α-ecdysone), supposed to be biologically inactive, and ecdysterone (20-hydroxyecdysone, crustecdysone, β-ecdysone) which exhibits hormonal activity. As yet it cannot be ruled out that ecdysone itself and related compounds, such as 3-dehydroecdysone, 3-dehydroecdysterone, 20,26-dihydroxyecdysone, could have a specific function in some particular processes regulating the moulting cycle[1-3]. Determinations of 'moulting hormone activity' in several insects has shown important and regular variations corresponding to the stage of development[4,5]. These determinations, carried out with the *Calliphora* bioassay, did not separate ecdysone from ecdysterone, both compounds being assayed together. In vivo experiments have shown that the hydroxylation rate of injected labelled ecdysone into ecdysterone is not constant, but that it also is strictly dependent on the physiological stage of the insects. In addition the excretion rate of ecdysone and ecdysterone with the faeces varies considerably during development[5].

These considerations have led us in the present study to investigate the influence of both the 20-ecdysone hydroxylase system and the rate of excretion on the titre of moulting hormone during the 5th (last) larval instar of *Locusta migratoria*.

Materials and methods. For the determination of the titre of ecdysone and ecdysterone, 15 g insects (*Locusta migratoria* in phase gregaria, reared as described in[5]) were homogenized in methanol and centrifuged. The supernatant was thin-layer chromatographed on silica gel (HF_{254}, Merck Darmstadt) with chloroform/methanol (80/20 vol/vol). Ecdysone and ecdysterone were eluted separately from the plates (identification under UV-light, reference substances being co-chromatographed in separate trails) and assayed in the *Calliphora* bioassay[6]. Hormone

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