were low, and as hormonal effect was observed only in the earliest incubation period, future incubations were carried out in a medium made of ROBINSON's buffer 96 parts: pooled rabbit serum 4 parts. This addition approximately doubled recoverable radioactivity and was used in all the studies herein reported.

Incubation was carried out in 25 ml capped flasks containing 5 ml of incubation medium. Flasks were gassed with O_2 at zero time and each 30 min thereafter. Hormone and/or antibiotic was included in the medium as indicated in the Table. After 30 min of preincubation, 0.2 μc of D-L-leucine-1-^{14}C of a specific activity of 5.24 mc/m*M* (New England Nuclear Corp.) was added to each flask. Incubation was at 37 ± 0.5 °C with shaking at 100 cycles/min. At the end of each incubation period, 5 ml of 10% trichloracetic acid (TCA) was added to the medium. Washing was repeated with TCA until the supernatant showed only background activity. The precipitate was digested in 0.4*N* NaOH for 12 h at 50 °C. Aliquots of 0.5 ml of the digest were counted, with background subtracted, in a Packard Model 3003 liquid scintillation counter in 15 ml of scintillation mixture. Quenching was determined to be insignificant in this system.

Significance of the difference between control and experimental means was examined by Student's *t*-test. *P*-values were taken from standard tables, with significance assigned at the level of $P < 0.05$.

Effect of aldosterone and actinomycin D on tissue incorporation of ^{14}C leucine

In medium	Cpm/mg wet tissue Control	Expl. medium	Expl. medium (% of control ± S.E.M.)	Incubation time (min)
Aldo[a] (12)[b]	24.5	45.8	187 ± 23.6[c]	30
Aldo (12)	43.9	57.9	132 ± 6.6[c]	60
Aldo (8)	56.2	91.0	162 ± 14.1[c]	90
Act D[c] (10)	12.0	10.1	83 ± 12.5	30
Act D (12)	22.7	16.8	74 ± 4.6[c]	60
Act D (8)	26.0	17.7	68 ± 5.1[c]	90
Aldo, Act D[d] (12)	45.8	29.3	64 ± 6.8[c]	30
Aldo, Act D (12)	57.9	49.8	86 ± 3.6[c]	60
Aldo, Act D (8)	91.0	58.8	64 ± 3.9[c]	90

[a] Aldo indicates experimental medium contained 4 μg/ml of D-aldosterone, control medium contained no hormone or antibiotic. [b] Numbers in parentheses indicate number of tissue samples in experimental groups, incubated in association with an equal number of control samples. [c] Act D indicates experimental medium contained 20 μg/ml of actinomycin D, control medium contained no hormone or antibiotic. [d] Aldo, Act D indicates experimental medium contained 4 μg/ml of D-aldosterone plus 20 μg/ml of actinomycin D, control medium contained 4 μg/ml of D-aldosterone. [e] Indicates a *p*-value less than 0.05.

The results show a significant increase in recoverable radioactivity in those tissue samples incubated in the presence of aldosterone. Actinomycin D, when included in the incubation medium with aldosterone, blocked the aldosterone stimulated increase in recoverable radioactivity. Actinomycin D also depressed recoverable radioactivity when administered alone among the 60 and 90 min samples.

In a small number of samples (4 incubated 30 min, 8 incubated 60 min, and 2 incubated 90 min), the inclusion of 50 μg/ml of puromycin in the medium containing aldosterone uniformly reduced recovered radioactivity to approximately $^1/_3$ of control levels. The enhancement of recoverable radioactivity in the tissue exposed to aldosterone suggests that the hormone is capable of stimulating a protein synthetic function in renal cortical tissue. This effect was blocked by inclusion of actinomycin D in the aldosterone containing medium.

As actinomycin D exerted some inhibitory influence on recoverable radioactivity in samples not exposed to exogenous aldosterone, the specificity of its inhibitory action may be questioned. Still, as endogenous aldosterone was not excluded from the system, it is possible that the effect of actinomycin D was specific for an aldosterone stimulated fraction of incorporation. The results are equivocal in this regard. That the majority of the recovered radioactivity did come from protein synthesized during incubation is suggested by the severe depression of recoverable radioactivity seen in those tissue samples incubated in the presence of puromycin[6].

Résumé. L'inclusion de D-aldostérone dans le milieu d'incubation a provoqué une accumulation élevée de l'acide amino-leucine dans la fraction protéique des tranches d'écorce du rein du lapin. Cette élévation a été arrêtée par des antibiotiques (actinomycine D et puromycine).

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In vivo Perfusion of Human Lung Tissue with 7α-^{3}H-Dehydroepiandrosterone ^{35}S-Sulfate. Metabolism of Steroid Conjugates. IV.

It appears to be well established that non-endocrine tissue also participates in the general metabolism of steroid hormones[1–5]. In continuation of previous experiments on the metabolism of steroid conjugates[6–9], the in vivo perfusion of human lung tissue with dehydroepiandrosterone (DHEA) sulphate was attempted.

In a 61-year-old male patient, undergoing a lobectomy due to a tumour in the upper lobe of his left lung, 5.05 μg 7α-^{3}H-DHEA ^{35}S-sulfate with 10.2×10^6 cpm ^{3}H and 2.37×10^6 cpm ^{35}S (^{3}H/^{35}S = 4.30) in physiological saline were continuously infused for 10 min into the branch of the pulmonic artery, leading to the lower lobe of the left

lung. From the corresponding pulmonic vein, ligated prior to the infusion, blood samples were withdrawn 2.5, 4, 4.5, 5.5, 6, 7, 9 and 10 min after the start of the infusion (L_1–L_8), yielding 4.0–5.0 ml of heparinized plasma. In addition, a sample of peripheral blood was taken 14 min after the beginning of the experiment and the urine collected over 24 h. Plasma samples from peripheral (P) or pulmonic vein blood were processed essentially as outlined in recent publications[10,11]. After extraction of free steroids with chloroform, total steroid conjugates were obtained by treatment with acetone and filtration of protein. The separation of lipophile steroid sulfatides, steroid sulfates and glucuronosides could be achieved by thin-layer chromatography of extracts on polyamide and silica gel[10]. Free steroids and steroids liberated from their conjugates by solvolysis or enzymatic hydrolysis were separated by repeated thin-layer chromatography in different solvent systems, diluted with non-labelled carrier and rechromatographed to constant specific ^3H-activity as free compounds and suitable derivatives, thus providing additional evidence for their identification. In a similar manner, the analysis of labelled steroids in the 24-h urine was performed.

As can be seen in Table I, the distribution of infused substrate in lung tissue proceeded rather slowly, causing a delayed appearance of labelled free or conjugated steroids in the venous effluent. The isolation of significant ^3H-activity from the fractions of free steroids in samples L_1–L_8 obviously reflects a certain sulfatase activity in human lung tissue. This is also confirmed by the fact that the $^3H/^{35}S$ ratio of sulfoconjugated steroids in these samples increased from an original value of 4.1 (for steroid sulfatides) or 4.3 (for steroid sulfates) to 6.2 and 5.6 respectively within 10 min, whereas the same fractions from peripheral plasma, collected in the 14th min, merely exhibited an isotope ratio of 4.8 and 4.5. At the same time, such findings reveal a partial resulfurylation of liberated steroids. On the other hand, the lack of significant ^3H-activity in the fraction of steroid glucuronosides in pulmonic vein blood should exclude the presence of glucuronosyl transferase activity in human lung tissue under such conditions. With regard to the metabolism of the substrate the percentage of the few isolated metabolites in the fraction of free steroids rose from approximately

Table I. Free and sulfoconjugated C_{19}- and C_{18}-steroids in pulmonic and peripheral vein plasma (per 5 ml)

Sample	Free $^3H/^{35}S$	r	Cpm in fraction			
			Sulfatide $^3H/^{35}S$	r	Sulfate $^3H/^{35}S$	r
L_1	43	>100	810	4.1	1,350	4.3
	0		198		324	
L_2	920	>100	22,900	4.5	8,000	4.3
	3		5,090		1,860	
L_3	1,590	>100	36,800	4.5	9,510	4.0
	0		8,160		2,350	
L_4	2,600	>100	34,200	4.6	7,600	4.8
	12		7,470		1,580	
L_5	2,290	>100	29,400	5.0	5,550	5.3
	8		5,850		1,040	
L_6	1,810	>100	23,700	5.6	3,870	5.3
	3		4,250		725	
L_7	1,620	>100	16,800	6.1	2,540	5.7
	0		2,760		444	
L_8	1,180	>100	12,900	6.2	1,210	5.6
	10		2,080		216	
P	46	>100	624	4.8	215	4.5
	0		129		48	

$r = \text{cpm}\,\frac{^3H}{^{35}S}$.

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Table II. C_{19}- and C_{18}-steroids isolated from combined sulfoconjugates of pulmonic and peripheral vein plasma (per 5 ml)

Steroid	Cpm ^3H in sample:								
	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	P
DHEA	784	21,900	18,600	16,500	13,800	9,120	7,040	3,870	322
Androstenediol	200	10,100	10,400	9,410	6,880	3,990	2,780	1,510	114
Androstenedione	14	94	480	1,420	690	189	117	160	2
Testosterone	68	408	1,220	1,370	773	472	357	110	3
Androsterone	8	108	304	570	786	284	105	102	108
Etiocholanolone	12	192	214	700	570	384	203	176	29
Estrone	6	35	44	65	58	24	20	30	7
Estradiol	8	72	70	106	82	35	42	24	3

DHEA, 3β-hydroxy-5-androstene-17-one; androstenediol, 5-androstene-3β17β-diol; androstenedione, 4-androstene-3,17-dione; testosterone, 17β-hydroxy-4-androstene-3-one; androsterone, 3α-hydroxy-5α-androstan-17-one; etiocholanolone, 3α-hydroxy-5β-androstan-17-one; estrone, 3-hydroxy-1,3,5-estratriene-17-one; estradiol, 1,3,5-estratriene-3,17β-diol.

20% in sample L_1 to more than 80% in sample L_8. In the fraction of sulfoconjugated steroids, consisting primarily of lipophile steroid sulfatides[6], the same metabolites accounted for little more than 40% of isolated ^{3}H-labelled compounds. 5-androstene-3β,17β-diol proved to be the most important metabolite, followed by 17β-hydroxy-4-androstene-3-one (testosterone) and 4-androstene-3,17-dione. The formation of labelled estrogens from sulfoconjugated DHEA in human lung tissue is not surprising in view of the observations of BRAMBILLA[12], who reported elevated estrogen levels in a case with a respiratory infection. As far as quantitative aspects are concerned, the direct metabolism[13] of sulfoconjugated DHEA by far exceeded the conversion of the substrate by indirect pathways, e.g. after hydrolysis of the conjugate. Although peripheral plasma and 24-h urine contained ^{3}H-labelled steroids, indicating their escape into the general circulation in the course of the experiment, the contribution of peripheral metabolism to the aforementioned results may be considered minor. For only 0.35% and 0.01% of infused ^{3}H-activity were detected in the fractions of urinary steroid sulfates and glucurono sides respectively. In contrast hereto, after the peripheral i.v. injection of the same substrate, 15–30% of administered ^{3}H-activity can be recovered from the 24-h urine[14].

Zusammenfassung. Bei der in-vivo Perfusion menschlichen Lungengewebes mit 7α-^{3}H-DHEA-^{35}S-sulfat zeigte es sich, dass im Lungengewebe sowohl Sulfatase- wie auch Sulfokinaseaktivität enthalten ist. Als Metaboliten konnten neben dem vorherrschenden 5-Androsten-3β,17β-diol auch Testosteron, Androstendion, Androsteron, Ätiocholanolon sowie Oestrogene nachgewiesen werden.

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Concerning Non-Darkening of Mutant *Habrobracon (Bracon hebetor)* Eyes as Consequence of a New Chromogen-Reducing Mechanism in Insect Larvae

The phenotypes of non-autonomous mutants are generally changed to wild-type if they feed on or receive through injections those substances of the normal pathway which lie behind the genetic block. Consequently, the eyes of *v*- and *cn*-pupae of *Drosophila* are found to be normally coloured when the larvae are fed with an extract from wild types[1]. Contrarily, the eyes of the orange-eyed mutant[2–4] of the parasitic wasp, *Habrobracon juglandis* (= *Bracon hebetor*), which cannot synthesize 3-hydroxykynurenine, do not darken although their larvae feed on the caterpillars of wild *Ephestia kühniella*. Present investigations have been aimed at providing an explanation for this unusual behaviour.

Haemolymph and fat body of *Ephestia* larvae contain small amounts of 3-hydroxykynurenine[5]. However, even when the hydroxykynurenine level of the *Ephestia* larvae is elevated by injecting the synthetic substance, no eye-darkening occurs in the parasite. Extraction of 3-day-old feeding larvae of *Habrobracon* (30 °C) reveals that hydroxykynurenine has not been completely metabolized by the host itself, because it was found in the gut and also to some extent in the haemolymph of *Habrobracon*. Moreover, compared with hydroxykynurenine, a high concentration of xanthurenic acid was found in haemolymph and gut.

After 2 days, eye pigmentation by ommochrome deposition begins in the wild-type prepupa. Extraction of the mutant prepupa, fed on hydroxykynurenine, reveals total absence of hydroxykynurenine in the haemolymph. Evidently within 2 days it is transaminated to xanthurenic acid and is excreted during moulting to the prepupa, leaving no hydroxykynurenine for ommochrome synthesis. In contrast to the wild type, the mutant spinning larvae and prepupae not fed with hydroxykynurenine accumulate kynurenine and kynurenic acid (Figure a, b). Figure c shows the third chemotype: the feeding and spinning larvae metabolized all hydroxykynurenine to xanthurenic acid before ommochrome synthesis in the eye began.

Now the question is why there is such a strong transamination in feeding and spinning larvae of *Habrobracon*. It is assumed that whenever toxic amounts of tryptophan are set free during development of insects they must be reduced to a compatible level[6,7], either by excretion[8] or by conversion into kynurenine and 3-hydroxykynurenine. These substances seem also to be incompatible in higher concentrations and hence are further metabolized to the ommochromes, specially in the pupal eyes of all insects after having induced, perhaps, the synthesis of the structural component of the ommochrome synthesizing apparatus[9]. In other stages or organs where structurally and enzymatically ommochrome synthesis is possible, colouring is always preceeded by chromogen accumulation, e.g. in the hibernating eggs of the silkworm[10], in larval skin and gonads of *Ephestia*[5], in haemolymph and fat body of *Cerura*[6] and in the MALPIGHIAN tubes of

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