loides and *U. lotrix* creates a strong possibility that, in these species at least, the structures have a function closely allied to that of the hair-pencils of Danaid butterflies. It is thus possible that (I) and (II) play a role in the mating of *Utetheisa* similar to that established for the related ketone (IV) in *Danaus gilippus berenice*[11].

Zusammenfassung. Die Duftorgane der männlichen Bärenspinner *Utetheisa pulchelloides* und *U. lotrix* (Fam. Arctiidae) scheiden Dihydropyrrolizine aus, die im Typ den Pheromonen von Schmetterlingen der Subfamilie Danainae (Fam. Papilionidae) gleichen. Wahrscheinlich handelt es sich um Derivate von Pyrrolizidin-Alkaloiden, die in den Wirtsplanzen der Raupen in hoher Konzentration vorkommen.

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Relationship Between Lipolysis and Storage of Diphenylhydantoin in the Adipose Tissue

In a previous paper it was reported that increased lipolysis results in a more marked storage of phenobarbital in adipose tissue[1]. Since diphenylhydantoin (DPH), an anticonvulsant drug, also accumulates in fat[2,3], it was considered important to establish if its uptake by adipose tissue is influenced by the degree of lipolysis.

Materials and methods. Male Sprague Dawley rats (average body weight 180 g) were used throughout all the experiments.

In vivo studies. DPH at 100 mg/kg body wt. was given s.c. to fed or fasted rats and the animals were sacrificed at different times after the administration. The concentration of DPH was measured in plasma and in adipose tissue (epididymal and perirenal).

In vitro studies. The study of the accumulation of DPH in adipose tissue was carried out according to the procedure previously described for phenobarbital[1]. The release of DPH from adipose tissue was studied according to the following procedure: epididymal adipose tissue, cut into small pieces and pooled, was preincubated for 1 h at 37 °C in Krebs Ringer phosphate at pH 7.4, containing 3% albumin and DPH 53 µg/ml. This adipose tissue (400–600 mg) preloaded with the drug was filtered, washed and added to 4 ml of medium, free of DPH, in the presence or absence of noradrenaline. The incubation was carried out at 37 °C, with gentle shaking, for 1 h and at the end of this period the DPH remaining in the adipose tissue was measured.

Determinations of DPH in 1 ml of plasma or 400 mg of adipose tissue were carried out according to the method of MORSELLI[4]; free fatty acids (FFA) were measured according to DOLE[5], with minor modifications. Triglycerides were determined according to VAN HANDEL, ZILVERSMIT and BOWMANN[6].

Results. DPH injected s.c. is rapidly absorbed in plasma reaching a peak at 30 min and 20 min, respectively, in fed and fasted rats. However, the rate of disappearance of this drug from plasma was quicker in fasted than in fed animals. Accumulation of DPH in both epididymal and perirenal adipose tissue was more rapid in fasted than in fed rats (Table I), although an interpretation of this phenomenon is difficult because of the different kinetics of plasma DPH in the two types of experimental conditions used.

The results obtained in vitro are more conclusive. Table II shows that DPH uptake by the adipose tissue of fed rats is proportional to the DPH concentration in the medium. Table III indicates that DPH accumulation in adipose tissue increases with the time of incubation. It was also found that the DPH content in adipose tissue was higher both per g of weight and per g of triglycerides, when lipolysis was increased by the presence of noradren-

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Table I. Levels of diphenylhydantoin (DPH) in plasma and adipose tissue after a single injection of DPH (100 mg/kg s.c.) to fed or to overnight fasted rats

Time[a] (min)	Fed rats DPH (µg/ml) or g ± S.E.			Fasted rats DPH (µg/ml) or g ± S.E.		
	Plasma	Adipose tissue		Plasma	Adipose tissue	
		Epididymal	Perirenal		Epididymal	Perirenal
10	13.5 ± 0.1	—	—	16.3 ± 0.3	—	—
20	16.2 ± 0.3	11.4 ± 0.6 (0.70)	12.0 ± 0.4 (0.74)	23.6 ± 1.8	7.7 ± 0.8 (0.32)	12.3 ± 0.3 (0.52)
30	18.4 ± 1.2	15.7 ± 1.1 (0.88)	15.6 ± 1.1 (0.84)	12.6 ± 0.9	21.3 ± 1.4 (1.69)	22.7 ± 1.2 (1.80)
60	16.3 ± 0.3	16.4 ± 0.9 (1.00)	16.4 ± 0.9 (1.00)	10.4 ± 1.2	26.1 ± 0.9 (2.50)	29.3 ± 1.1 (2.81)
120	12.6 ± 1.6	25.1 ± 1.4 (1.99)	25.5 ± 1.1 (2.04)	9.3 ± 0.1	10.9 ± 1.2 (1.17)	12.7 ± 0.1 (1.36)
240	3.6 ± 1.4	9.9 ± 3.7 (2.75)	9.7 ± 0.4 (2.69)	8.1 ± 0.5	—	—
360	0.1 ± 0.0	—	—	1.2 ± 0.1	—	—

[a] Time elapsed between DPH administration and sacrifice of the animals. In brackets: the ratio between adipose tissue and plasma levels of DPH.

Table II. Accumulation in epididymal adipose tissue of DPH added to the incubation medium

Addition to the medium (μg/ml)	Incubation time (h)	FFA (μEq/g/h) ± S.E.	TG (g/100 g/AT) ± S.E.	DPH	
				(μg/g) AT ± S.E.	(μg/g) TG ± S.E.
DPH 50	1	0.79 ± 0.06	59.5 ± 1.5	63.3 ± 5.9	106
DPH 100	1	0.76 ± 0.03	56.9 ± 2.7	145.5 ± 4.7	255

Medium: phosphate buffer pH 7.4 + bovine albumin (fract V.) 3%. AT, epididymal adipose tissue obtained from fed rats; DPH, diphenylhydantoin; FFA, free fatty acids; TG, triglycerides.

Table III. Accumulation of DPH in adipose tissue in conditions of basal or noradrenaline-stimulated lipolysis

Incubation time (h)	Additions to the medium DPH (50 μg/ml)			DPH (50 μg/ml) + NA (0.25 μg/ml/h)		
	FFA (μEq/g) AT	DPH (μg/g) AT	DPH (μg/g) TG	FFA (μEq/g) AT	DPH (μg/g) AT	DPH (μg/g) TG
1	2.4 ± 1.5	55.8 ± 5.3	106	8.8 ± 0.3	65.0 ± 4.6	116
2	3.1 ± 0.2	63.8 ± 2.8	106	21.2 ± 0.3	87.0 ± 6.9	136
3	1.6 ± 0.1	78.8 ± 6.7	147	24.9 ± 1.0	106.4 ± 1.0	169

AT, epididymal adipose tissue; DPH, diphenylhadantoin; FFA, free fatty acids; NA, noradrenaline bitartrate (0.25 μg/ml) were added every h during the incubation; TG, triglycerides.

Table IV. Retention of DPH in adipose tissue during basal or noradrenaline-stimulated lipolysis

Addition to the medium (μg/ml)	DPH (μg/g) AT ± S.E.		FFA (μEq/g) AT
	0 min	60 min	60 min
—	71.3 ± 0.1	31.9 ± 1.9	4.1 ± 0.3
NA 0.25	71.3 ± 0.1	36.9 ± 1.1	9.2 ± 0.6

Epididymal adipose tissue (AT) was previously incubated for 1 h in a medium containing 53.3 μg/ml of diphenylhydantoin (DPH), then the adipose tissue was filtered, washed and incubated in a medium free of DPH with or without noradrenaline (NA). FFA, free fatty acids.

aline. When DPH was removed from the medium, the degree of its retention by adipose tissue was relatively greater in the presence of increased lipolysis due to noradrenaline action (Table IV).

Conclusions. When diphenylhydantoin (DPH) is administered to rats, its accumulation and disappararance rate in and from both plasma and adipose tissue is different in fed and fasted animals. This suggests that the degree of lipolysis, which is increased during fasting, may exert a role in the storage of DPH in adipose tissue.

Experiments carried out in vitro show, in fact, that DPH accumulates more extensively in epididymal adipose tissue when lipolysis is stimulated by noradrenaline, as compared to the basal condition. Part of this effect may be related to an increased retention of DPH by adipose tissue during a high rate of lipolysis. Since a high rate of lipolysis involves an increased release of FFA in the medium, one may speculate that DPH is displaced from its albumin bounds, so that it is able to enter into adipose tissue.

The findings obtained with DPH are in substantial agreement with previous results obtained on the accumulation of phenobarbital in adipose tissue, in relation to lipolytic activity[1]. In this respect, DPH and phenobarbital behave differently from corticosterone whose uptake by adipose tissue decreases during a high rate of lipolysis[7].

Riassunto. La cinetica di accumulo della difenilidantoina nel tessuto adiposo é diversa negli animali a digiuno o normalmente alimentati. Esperimenti in vitro mostrano che il tessuto adiposo epididimale quando é stimolato con noradrenalina, che aumenta la lipolisi, accumula la difenilidantoina e la trattiene in maggior quantità di quando é in condizione di lipolisi basale.

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