Biochemical Pharmacology, Vol. 34, No. 20, pp. 3683–3688, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

UPTAKE *IN VITRO* OF LIPOPHILIC MODEL COMPOUNDS INTO ADIPOSE TISSUE PREPARATIONS AND LIPIDS

CRISTINA DI FRANCESCO and MARCEL H. BICKEL
Department of Pharmacology, University of Berne, CH-3010 Berne, Switzerland

(*Received* 1 *March* 1985; *accepted* 20 *May* 1985)

Abstract—*In vitro* uptake of 11 lipophilic model compounds into rat epididymal adipose tissue slices, adipocytes, triglycerides, and lecithin was studied. Relative uptake at equilibrium into adipose tissue slices increased from 6 to 87% in the following sequence: phenazone, morphine < pentobarbital < glutethimide, phenylbutazone < thiophental, methadone < chlorpromazine, imipramine. In the presence of albumin a similar sequence was obtained at lower uptake levels, with DDE and 2,4,5,2′,4′,5′-hexachlorobiphenyl (6-CB) on top with 95% uptake. However, the time to reach equilibrium was unproportionately greater for DDE and 6-CB (16–40 hr) than for other compounds (1–4 hr). A linear positive correlation was found between relative uptake and partition coefficient (octanol/water). Relative uptake was independent of drug concentration. There were no significant differences between uptake values measured with adipose tissue slices, adipocytes, triolein, and a saturated short-chain triglyceride. In contrast, uptake into lecithin was not correlated with the octanol partition coefficient. Thiopental, imipramine, and 6-CB were taken up into lean tissue slices (liver, lung, skin) in excess of their lipid content, suggesting additional binding sites. Release from preloaded adipose tissue slices followed first order kinetics, was accelerated by albumin, and was much slower for 6-CB and DDE than for thiopental and imipramine.

The results indicate that uptake of lipophilic xenobiotics *in vitro* is a partition process between the aqueous medium and the triglyceride of the adipose tissue preparation. In contrast, the extent of adipose tissue storage of drugs *in vivo* has recently been shown not to correlate with octanol partition coefficients.

Adipose tissue is known to act as a storage compartment for many drugs. It may therefore control the pharmacokinetics and thus the pharmacodynamics of certain drugs [1, 2]. The well-known examples of drugs stored in adipose tissue are thiopental [2, 3] and polychlorinated hydrocarbons like the DDT-type insecticides [4, 5]. These observations were made in the 1950s and led to the widespread and persistent belief that high lipid solubility of a drug results in a preferential, extensive accumulation in adipose tissue. However, this generalization is becoming questionable. As a matter of fact, an appreciable number of drugs, which are more lipophilic than thiopental, does not enter adipose tissue in significant amounts *in vivo*. This is the case with chlorpromazine [6, 7], imipramine [7–9], and other basic drugs [10–16]. In addition, the pattern of redistribution from blood to lean tissues and to adipose tissue is qualitatively the same for thiopental, DDE, and 2,4,5,2′,4′,5′-hexachlorobiphenyl (6-CB); yet DDE, 6-CB, and related highly lipophilic compounds, which rapidly invade lean tissues, are extremely slow to enter adipose tissue which eventually stores high amounts of them [17–20]. These findings indicate that adipose tissue storage of drugs is not simply a matter of lipid solubility and partition and that the lipophilicity concept of adipose tissue storage has to be re-evaluated [21]. Such a re-evaluation should also include the study of drug uptake into adipose tissue preparations *in vitro*. Information on this uptake process is hardly available, even though its comparison with the storage *in vivo* would help to interpret the fact that certain lipophilic drugs are stored in adipose tissue whereas others are not. If uptake *in vitro* parallels storage *in vivo*, then permeation into adipose tissue may govern adipose tissue storage *in vivo*. If, on the other hand, storage *in vivo* of individual drugs differs from their uptake *in vitro*, this could suggest that the former is governed by factors present in vivo only and outside adipose tissue.

In this work we report on uptake and its kinetics *in vitro* of lipophilic model compounds into adipose tissue preparations and pure lipids. The 11 model compounds used vary widely in their chemical structures and lipophilicities (log *P* octanol/water 0.2–6.7). Uptake was studied with rat adipose tissue slices, isolated adipocytes, triglycerides, and lecithin. The results were compared with adipose tissue invasion kinetics and storage *in vivo*.

MATERIALS AND METHODS

Chemicals and drugs. The following unlabelled drugs were obtained: imipramine, glutethimide, phenylbutazone from Ciba–Geigy (Basel, Switzerland); chlropromazine (Bayer, Leverkusen, F.R.G.); thiopental (Abott, Zug, Switzerland); pentobarbital, morphine (Siegfried, Zofingen, Switzerland); 1,1-bis-(*p*-chlorophenyl)-2,2,2-trichloroethane (DDT, 98%), (Fluka, Buchs, Switzerland); 1,1-bis-(p-chlorophenyl)-2,2-dichloroethene (DDE, 99%), (EGA-Chemie, Steinheim, F.R.G.); 2,4,5,2′,4′,5′-hexachlorobiphenyl (6-CB), (Analabs, North Haven, CT). Labelled drugs: ^{14}C-glutethimide, ^{14}C-phenylbutazone (gifts from Ciba–Geigy); ^{14}C-pentobarbital, ^{14}C-6-CB, ^{14}C-phenazone (NEN, Du Pont, U.K.); ^{14}C-thiophental

(ICN, Irvine, CA); ^{14}C-imipramine, ^{14}C-morphine, ^{14}C-DDT (The Radiochemical Centre, Amersham, U.K.); ^{14}C-chlorpromazine (Applied Science Labs., State College, PA); ^{14}C-methadone (CEA, Gif-sur-Yvette, France). ^{14}C-DDE was prepared by chemical dehydrochlorination of ^{14}C-DDT according to Apple [22].

Bovine serum albumin, fraction V (BSA), (Miles Scientific Naperville, IL); collagenase from *U. histolyticum* (Worthington Biochem. Corp. Freehold, NJ); Insta-Gel II liquid scintillation cocktail, Soluene 350 tissue solubilizer (United Technologies Packard, Zurich, Switzerland). All other chemicals and drugs were standard commercial products obtained from Fluka (Buchs, Switzerland) or from Merck (Darmstdt, F.R.G.). Modified phosphate–Krebs–Ringer buffer, pH 7.40, was prepared according to Rodbell [23]. In specified cases the buffer contained 4% albumin, dialysed against buffer. pH-values other than 7.4 were adjusted with NaOH or HCl and the osmolarity controlled.

6-CB, DDE, and glutethimide were dissolved in hexane or ethanol, the solvent evaporated under nitrogen, and buffer containing albumin was added. The sample was then continuously agitated with a magnetic stirrer at room temperature for about 17 hr. Before use the solution was centrifuged for 10 min at 12,000 *g*. The radioactivity of the supernatant was measured and the effective concentration of the solution calculated. All other drugs were directly dissolved in buffer with or without albumin.

Adipose tissue preparations. Male SIV 50-rats (Institut für Zuchthygiene, Zurich) of 200–300 g body weight were used.

Epididymal fat pads were cut in slices of 5–200 mg and kept in buffer at 4° until use, never exceeding half a day.

Adipocytes were prepared according to Rodbell [23]. Pieces of 70 mg epididymal adipose tissue were incubated in borosilicate glass tubes with 250 μl of buffer containing 4% albumin, 3.4 mg/ml collagenase, and 3 mM glucose for 1.5 hr at 37° in a metabolic shaker. The resulting cell suspension was centrifuged for 1 min at 400 *g*. The sedimented cells were removed by aspiration while the floating adipocytes were washed by suspending them in 500 μl buffer. This procedure was repeated twice. The yield was about 90% as shown by comparison of the lipid contents of tissue and isolated adipocytes (see analytical procedure).

Solutions of the isolated tissue lipids were added to borosilicate glass tubes and the organic solvent was evaporated under nitrogen; egg yolk lecithin was put in borosilicate glass tubes and the medium added before incubation. Triolein was added on top of the aqueous medium with a Pasteur pipette.

Skin preparations were obtained from rat abdominal skin freed from hair and subcutaneous tissues with a lancet.

Uptake and release studies. Tissue slices, adipocytes, or lipids were incubated with 1 ml of drug solution in closed borosilicate glass tubes in a metabolic shaker at 37° for the time selected. Slices were then mechanically removed, adipocytes floated for 5 min at 400 *g*, and triolein aspirated. The medium was aspirated for analysis with a syringe fitted with a millipore filter (0.45 μm). Controls lacking the biological preparation or lipid were run.

For release experiments, epididymal adipose tissue slices were incubated as described, until uptake equilibrium was reached. The slices (50 mg/ml) were then transferred to drug-free medium which was changed every 30 min. This procedure was repeated 8 times, and the drug concentrations in the individual release media was determined. The logarithm of amount in tissue was plotted against time, and half-lives were calculated from linear regressions.

Analytical procedures. The drug concentration in the medium after incubation was determined by liquid scintillation counting. The amount left in the tissue was calculated as the difference between total amount and amount in medium. This procedure is justified by the balances of 93 ± 5% (N = 8) obtained with drug determination in tissue. In addition, the control incubations allowed correction for adsorption and evaporation.

For the determination of lipid contents the samples were extracted according to Renkonen *et al.* [24] and the lipid content assayed gravimetrically. The same extraction procedure was used for the isolation of lipids from biological materials. Lipids were then dissolved in chloroform/methanol (5:8) at a concentration of 10 mg/ml and kept at −20° until used.

RESULTS

Uptake studies

The uptake into adipose tissue preparations and lipids of 11 lipophilic model compounds which vary in their chemical structures and lipophilicities was investigated. Kinetics of uptake into adipose tissue slices (% of amount of drug in tissue) is shown in Fig. 1. In order to include highly lipophilic compounds like 6-CB and DDE which are extremely insoluble in water, the incubation medium was supplemented with 4% serum albumin. These two xenobiotics were much slower to reach equilibrium than the others. At standard conditions (see Materials and Methods), the uptake of 6-CB into adipocytes is faster than into adipose tissue slices, whereas for triolein the uptake kinetics is even slower than for tissue slices. However, uptake into triolein can be dramatically accelerated by vortexing the incubations which increases relative uptake after 5 min from <5% to 45%, and after 60 min from 21% to 81%. The presence of 4% albumin reduces relative uptake at equilibrium of imipramine from 87% to 57%, and of thiopental from 71% to 29%. Relative uptake is also a function of adipose tissue concentration. Increasing the number of tissue slices per incubation increased the equilibrium level. All values of relative uptake given in the following are equilibrium values obtained at a tissue concentration of 50 mg/ml, unless otherwise stated.

Relative uptake was not dependent on drug concentration over a tested range of three orders of magnitude (Fig. 2). This was even so at very low tissue concentrations (2.5 or 5 mg/ml) as shown for imipramine (Table 1). The distribution coefficient P' (drug concentration ratio tissue/medium) was independent of the concentrations of both drug and

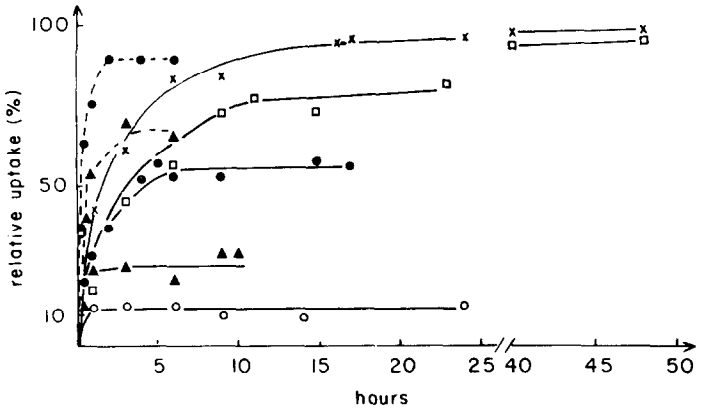

Fig. 1. Kinetics of uptake of model compounds into adipose tissue slices. Rat adipose tissue slices (50 mg/ml) were incubated at 37° with drug in buffer (pH 7.4) containing 4% BSA. Broken lines show uptake in the absence of BSA. Each point is the mean of 2–3 incubations. (×) DDE, (□) 6-CB, (●) imipramine, (▲) thiopental, (○) pentobarbital.

tissue. For imipramine log P' was 2.25 ± 0.1 (Table 1).

The relative uptake values of the model compounds into adipose tissue slices in the absence of albumin are given in Table 2. In Table 3 relative uptake is also shown for adipocytes and pure lipids. Since these experiments also include 6-CB and DDE they were carried out in the presence of albumin. The relative amounts of drug taken up into adipose tissue slices, adipocytes, and triglycerides was highest for 6-CB and DDE and decreased in the order: chlorpromazine, methadone, imipramine > thiopental, glutethimide > pentobarbital, phenazone, morphine, phenylbutazone. The depression of relative uptake by the competing binder, albumin, emerges from the comparison of Tables 2 and 3. As shown in Fig. 3 there is a linear correlation between the relative uptake into adipose tissue slices (or adipocytes or triglycerides) and log P (partition coefficient octanol/water) of the model compounds. This is even so in the presence of albumin. The correlation for adipose tissue slices can be expressed by the following equations: In the absence of albumin, relative uptake U_{rel} (%) = 17.5 × log P' + 3, and in the presence of 4% albumin U_{rel} (%) = 15.4 × log P − 11. In the presence of albumin only the very strongly bound phenylbutazone drops out of the correlation.

The following findings would also suggest that uptake into adipose tissue *in vitro* is a partition. Relative uptake of imipramine was unchanged when adipose tissue slices were boiled, stored at 4° for up to 6 days, or when the surface/mass ratio of the slices was varied. Imipramine uptake was increased by increasing pH, whereas thiopental showed the opposite behaviour. In contrast to adipose tissue slices, adipocytes, and tryglycerides, the uptake capacity of lecithin decreased in a sequence which is not correlated to log P: imipramine, chlorpromazine, DDE > pentobarbital, glutethimide, 6-CB, thiopental, morphine > phenylbutazone (Table 3).

In order to check whether uptake into non-adipose tissues is due to their lipid content, relative uptake was compared in tissue slices and in the corresponding amount of lipids extracted from these tissues. Skin and liver took up more 6-CB and thiopental than the corresponding lipids, and lung took up more imipramine.

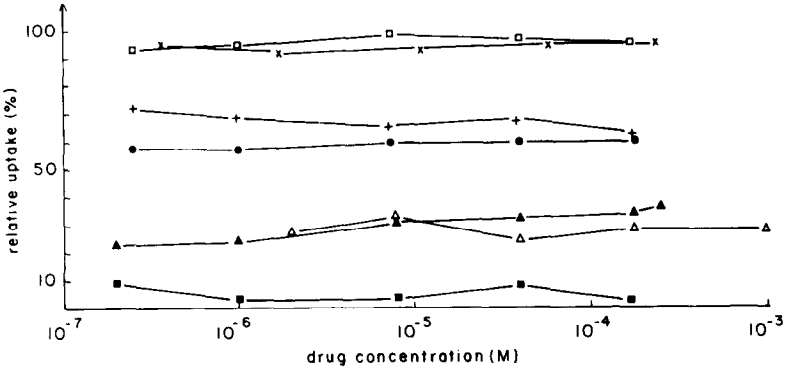

Fig. 2. Uptake of model compounds at varying concentrations into adipose tissue slices in the presence of albumin. Slices (50–70 mg/ml) were incubated until equilibrium at 37° in buffer (pH 7.4) containing 4% BSA. Each point is the mean of 3 or more experiments. (×) DDE, (□) 6-CB, (+) chlorpromazine, (●) imipramine, (▲) thiopental, (△) glutethimide, (■) morphine.

Table 1. Uptake of imipramine into rat adipose tissue slices: effect of tissue and drug concentrations on relative uptake and distribution coefficient P'

Concentration					
Adipose tissue (mg/ml)	Drug initial (μmol/ml)	Drug in adip. tissue (μmol/ml)	Drug in medium (μmol/ml)	Relative uptake (%)	P'*
2.5	10^{-3}	1.48×10^{-1}	6.3×10^{-4}	37	235
2.5	10^{0}	1.32×10^{2}	6.7×10^{-1}	33	197
5.0	10^{-3}	1.06×10^{-1}	4.7×10^{-4}	53	231
5.0	10^{0}	8.8×10^{1}	5.6×10^{-1}	44	157
50	10^{-3}	1.74×10^{-2}	1.3×10^{-4}	87	134
50	10^{0}	1.76×10^{1}	1.2×10^{-1}	88	147

* Drug concentration ratio tissue/medium.

Slices were incubated at 37° until equilibrium (5 hr) in buffer pH 7.4 in the absence of albumin. Each value represents the mean of 3 experiments. Density of adipose tissue was not taken into consideration.

Release experiments

Release from preloaded adipose tissue slices of a selection of model compounds could be characterized by the following results. The presence of 4% albumin in the medium accelerated release. An increase in the concentration of tissue enhanced the half-life of release. Table 4 shows the wide variations in release half-lives, the sequence being 6-CB > DDE > imipramine > thiopental. For thiopental and imipramine the release half-lives were independent of their initial concentrations in the tissue over a 10-fold range, thus confirming a first-order release. 6-CB was released much faster from skin than from adipose tissue slices (table 4).

Table 2. Uptake of model compounds into adipose tissue slices in the absence of albumin

Compound	Relative uptake (%)	N
Imipramine	87 ± 2	16
Chlorpromazine	85 ± 5	12
Methadone	75 ± 3	9
Thiopental	71 ± 3	6
Phenylbutazone	55 ± 3	5
Glutethimide	48 ± 5	7
Pentobarbital	29 ± 4	7
Morphine	8 ± 4	7
Phenazone*	5 ± 6	15

Means ± S.D. N = number of experiments. Slices (50 mg/ml) were incubated at 37° with drug (5 μM) in buffer pH 7.4 until equilibrium.

* Antipyrine.

DISCUSSION

Adipose tissue storage of drugs *in vivo* has recently been shown not to correlate with lipophilicity [21]. Uptake of drugs into isolated adipose tissue preparations and pure lipids was therefore studied in this work with the aim to draw conclusions from discrepancies between the behaviour of drugs *in vitro* and *in vivo*.

Relative uptake under equilibrium conditions of 11 model compounds was determined, using a variety of preparations and conditions. All the following

Table 3. Uptake of model compounds into adipose tissue preparations and pure lipids in the presence of 4% albumin

Compound	Relative uptake (%)				
	Adipose tissue slices	Adipocytes	Glyceroltrioleate	Glyceroltriheptanoate	Lecithin
DDE	95 ± 2 (31)		98 ± 1 (15)	97 ± 10 (8)	87 ± 9 (14)
6-CB	94 ± 5 (9)	99 ± 0.5 (5)	90 ± 2 (6)	91 ± 9 (6)	45 ± 12 (22)
Chlorpromazine	68 ± 6 (14)		76 ± 8 (6)	86 ± 9 (6)	89 ± 3 (5)
Methadone	60 ± 2 (9)				
Imipramine	57 ± 6 (27)	62 ± 8 (18)	68 ± 5 (27)		91 ± 6 (15)
Thiopental	29 ± 7 (37)	25 ± 6 (10)	27 ± 3 (13)		37 ± 8 (18)
Glutethimide	28 ± 3 (15)		31 ± 6 (8)	45 ± 7 (8)	54 ± 4 (6)
Pentobarbital	10 ± 8 (32)		17 ± 8 (6)		55 ± 5 (9)
Phenazone	6 ± 4 (15)				
Morphine	5 ± 3 (15)		5 ± 5 (6)		25 ± 7 (12)
Phenylbutazone	0 ± 7 (6)		4 ± 5 (9)		4 ± 3 (8)

Means ± S.D. In brackets number of experiments. Biological preparations or lipids (50 mg/ml) were incubated at 37° with drug (2.5×10^{-7} to 10^{-3} M) in buffer pH 7.4 until equilibrium. Relative uptake is independent of drug concentration (see Fig. 2).

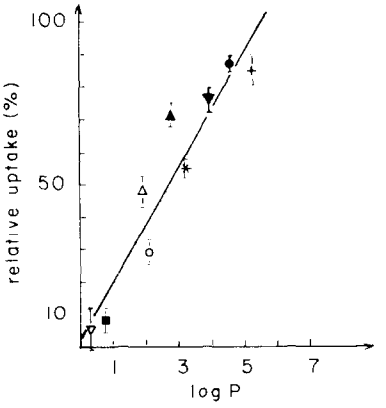

Fig. 3. Correlation between uptake into adipose tissue slices and partition coefficients (octanol/water) of compounds. Slices (50 mg/ml) were incubated until equilibrium at 37° in buffer (pH 7.4) without BSA. Each point is the mean ± S.D. of 5–16 experiments. Log *P*-values (octanol/water) were taken from Hansch and Leo [25]. (▽) phenazone, (■) morphine, (○) pentobarbital, (△) glutethimide, (*) phenylbutazone, (▲) thiopental, (▼) methadone, (●) imipramine, (+) chlorpromazine.

results are indicative that uptake can be considered as a partition between the aqueous and adipose phase. Relative uptake was a function of adipose tissue concentration, but not of drug concentration, and the distribution coefficient (tissue/medium) was independent of either concentration (Fig. 2, Table 1). The uptake values obtained with adipose tissue slices, adipocytes, and pure triglycerides showed no significant differences (Table 3). Most important, even though chemically widely different compounds were used, a positive linear correlation emerged between relative uptake and partition coefficient octanol/water (Fig. 3). Furthermore, stored or boiled adipose tissue slices showed unchanged uptake. Variations in shape and consequently in surface of adipose tissue slices did not influence uptake either. This observation is important in view of the fact that adipose tissue slices cannot be standardized with respect to thickness.

Whereas relative uptake at equilibrium of 11 model compounds was found to be correlated with lipophilicity, such a correlation did not exist with respect to the rate of uptake (Fig. 1). The most lipophilic members, 6-CB and DDE, took more than 15 hr to reach their distribution equilibrium, while the other compounds took 1–4 hr. The unproportionately slow invasion into adipose tissue of 6-CB is reminiscent of the situation *in vivo* [17, 18, 20]. A permeation barrier is unlikely to be the cause of this slow uptake, as the uptake into pure triolein, i.e. a system without a biological membrane, is even slower than into adipose tissue slices. Diffusion problems of 6-CB, which is almost totally albumin-bound in the medium, may explain the slow uptake, since the uptake of 6-CB into well dispersed adipocytes is faster than the uptake into tissue slices. Furthermore, the uptake into triolein can be drastically accelerated by vortexing the incubations. However, serum albumin which was used to solubilize 6-CB and DDE, slowed and decreased the relative uptake of the other model compounds by 20–65% (Fig. 1, Tables 2 and 3), and phenylbutazone, which is very strongly bound to albumin, was not taken up at all in the presence of 4% albumin. Correspondingly, release was accelerated by the presence of albumin in the medium (Table 4). The compounds with a rapid uptake were also rapidly released, and slow-uptake compounds (6-CB, DDE) were slowly released (Table 4).

The observation that each compound showed a comparable uptake value with adipose tissue slices, adipocytes, and triglycerides (Table 3) suggests that the triglyceride content of the adipocytes is the storage medium of adipose tissue. Chain length and degree of saturation of the fatty acid moieties do not seem to influence uptake.

The observation that non-adipose tissues take up more thiopental, imipramine, or 6-CB than would correspond to their lipid content suggests the presence of additional binding sites in lean tissues. This would indeed by a prerequisite for a binding competition between adipose and lean tissues *in vivo*. Skin may be an extreme case as it acts as an alternative storage tissue for 6-CB [17, 18, 26], yet it is devoid of triglycerides [18]. It is also remarkable that *in vivo* 6-CB appears much faster in liver than it does in adipose tissue [17, 18, 20].

In contrast to adipose tissue preparations and pure triglycerides the relative uptake into lecithin is not correlated with the partition coefficient. Typically, the basic lipophilic drugs, imipramine and chlorpromazine, show the highest uptake into lecithin, and uptake of the basic but more polar morphine is

Table 4. Half-lives of release of model compounds from slices of rat adipose tissue and skin

Compound	Adipose tissue			Skin		
	Serum albumin			Serum albumin		
	0%	4%	N	0%	4%	N
Thiopental	69 ± 8 min	40 ± 15 min	3–4			
Imipramine	205 ± 56 min	120 ± 14 min	3–4			
6-CB	363 ± 129 hr	30 ± 2 hr	5–6	22 hr	3.0 hr	2
DDE	92 ± 7 hr	32 ± 3 hr	4			

N, number of experiments. Means ± S.D. Procedure see Materials and Methods. Tissue concentration: 50 mg/ml.

5 times higher than into triglyceride preparations (Table 3). This is in agreement with the fact that these basic lipophilic drugs interact with phospholipids [27] whereby their polar headgroups are also involved [28].

In conclusion, uptake of xenobiotics into adipose tissue preparations *in vitro* is a function of the lipophilicity of the compound and thus a partition process. In contrast, the degree of adipose tissue storage *in vivo* is not correlated with partition coefficients. Thus, the more complex situation *in vivo* must be due to factors outside adipose tissue, i.e. to the presence of lean tissues. Extent and kinetics of adipose tissue storage *in vivo* seem to be mainly controlled by a competition of binding of the drugs to well-perfused lean tissues (which is dependent on chemical structure) and partitioning into poorly perfused adipose tissue (which is dependent on lipophilicity). A physiologic pharmacokinetic model based mainly on flow limitation and binding competition has been recently proposed [21] and is likely to be able to explain the behaviour of prototypes like thiopental, imipramine, 6-CB, and others. Elimination rate, which is not of primary importance [21], is also included in this model.

Acknowledgements—We wish to thank the Swiss National Science Foundation for support; Miss B. Zbinden and Mr F. Hafen for technical assistance.

REFERENCES

1. B. B. Brodie and C. A. M. Hogben, *J. Pharm. Pharmac.* **9**, 345 (1957).
2. L. C. Mark, *Handbook exp. Pharmacol.* **28/1**, 258 (1971).
3. B. B. Brodie, L. C. Mark, E. M. Papper, P. A. Lief, E. Bernstein and E. A. Rovenstine, *J. Pharmac. exp. Ther.* **98**, 85 (1950).
4. W. J. Hayes, Jr., *Ann. Rev. Pharmac.* **5**, 27 (1965).
5. W. F. Durham, *Ann. N. Y. Acad. Sci.* **160**, 183 (1969).
6. N. P. Salzman and B. B. Brodie, *J. Pharmac. exp. Ther.* **118**, 46 (1956).
7. M. H. Bickel, B. E. Graber and M. Moor, *Life Sci.* **33**, 2025 (1983).
8. J. V. Dingell, F. Sulser and J. R. Gillette, *J. Pharmac. exp. Ther.* **143**, 14 (1964).
9. M. H. Bickel and R. Gerny, *J. Pharm. Pharmac.* **32**, 669 (1980).
10. E. Eschenhof and J. Rieder, *Arzneimittel-Forschung* **19**, 957 (1969).
11. S. M. Huang and W. L. Chiou, *J. Pharmacokin. Biopharm.* **9**, 711 (1981).
12. B. Dubnick, C. A. Towne, J. M. Hargifan and G. E. Phillips, *Biochem. Pharmac.* **17**, 1243 (1968).
13. J. C. Rickards, G. E. Boxer and C. C. Smith, *J. Pharmac. exp. Ther.* **98**, 380 (1950).
14. A. L. Misra, R. Bloch, N. L. Vadlamani and S. J. Mulé, *J. Pharmac. exp. Ther.* **188**, 34 (1974).
15. S. M. Hess, P. A. Shore and B. B. Brodie, *J. Pharmac. exp. Ther.* **118**, 84 (1956).
16. F. Marcucci, E. Mussini, R. Airoldi, R. Fanelli, A. Frigerio, F. de Nadai, A. Bizzi, M. Rizzo, P. L. Morselli and S. Garattini, *Clin. Chim. Acta* **34**, 321 (1971).
17. H. B. Matthews and M. W. Anderson, *Drug Metab. Disposit.* **3**, 371 (1975).
18. S. Mühlebach and M. H. Bickel, *Xenobiotica* **11**, 249 (1981).
19. I. G. Sipes, M. L. Slocumb, D. F. Pery and D. E. Carter, *Toxic. appl. Pharmac.* **62**, 264 (1982).
20. S. Mühlebach, P. A. Wyss and M. H. Bickel, *Xenobiotica* **15**, in press (1985).
21. M. H. Bickel, *Progr. Drug. Res.* **28**, 273 (1984).
22. E. J. Apple, Thesis University of Arizona (1968).
23. M. Rodbell, *J. biol. Chem.* **239**, 375 (1964).
24. O. Renkonen, T. U. Kosunen and O. V. Renkonen, *Ann. Med. Exp. Fenniae (Helsinki)* **41**, 375 (1963).
25. C. Hansch and A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*. John Wiley, New York (1979).
26. P. A. Wyss, S. Mühlebach and M. H. Bickel, *Drug. Metab. Disposit.* **10**, 657 (1982).
27. C. di Francesco and M. H. Bickel, *Chem.-Biol. Interactions* **16**, 335 (1977).
28. J. Römer and M. H. Bickel, *Biochem. Pharmac.* **28**, 799 (1979).