To further evaluate the hypothesized effects of anthocyanins, flower petals were collected, and young larvae were allowed to feed on them in the laboratory (4 replicates of 50 insects, 4 chemical analyses per data point). Table 2 presents tannin, gossypol, and chrysanthemin concentrations of white and red petals of 2 glanded and 2 non-glanded varieties, and for tobacco budworm 5-day larval weights and percent survival. Larvae fed more successfully on white, 1st day petals then on red, 2nd day petals, and also more successfully on non-glanded petals than on glanded petals. The tannin contents of the non-glanded white petals were higher than those of the glanded strains, but there was little difference in the red petal tannin contents. The gossypol content of the petals of the glanded strains was much higher than those of the non-glanded strains, and the chrysanthemin content of red petals was also much higher than those of white petals as expected. Based collectively on data in table 2 and figure 1, we suggest that chrysanthemin is more important than tannin for the reduced larval size and survival on glandless (gossypol-low) red petals. Gossypol and chrysanthemin contribute to the toxicity of glanded red petals and gossypol to that of glanded white petals. We now have preliminary data that larvae fed leaves and bracts of red cottons gained 20% less (statistically significant) than those fed leaves and bracts from comparable green strains. Thus, red coloration now appears to be a factor of considerable importance in insect feeding on both petals and leaves. There is still a residual mortality of insects feeding on white glandless petals (table 2). This can be attributed at least in part to the flavonoids, some of which we have previously identified[17] and demonstrated to be toxic to this insect (table 1).

We were able to observe by magnification that tobacco budworm larvae avoid pigment glands during feeding. We are also able to observe by means of 5 µm paraplast section through a young expanding cotton leaf of cultivar DH-126 at the pigment gland site, the outer anthocyanin containing envelope (halo) surrounding the pigment gland (fig. 2). In fresh tissue sections, this outer halo stains bright red in acid, and green when neutralized with base, verifying that it is anthocyanin. The feeding deterrence of the pigment gland which has been attributed to gossypol may therefore be due at least in part to the anthocyanin halo.

1 In cooperation with the Mississippi Agriculture and Forestry Experiment Station, Mississippi State. Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
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A technique for the comparison of biological distribution and solvent partition of drugs[1]

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Summary. A dialysis technique is described which allows the measurement of drug distribution between buffer and solvents as well as between buffer and biological preparations under identical experimental conditions. Partition and distribution coefficients of thiopental, pentobarbital, imipramine, and chlorpromazine were determined using octanol, other solvents, and tissue homogenates.

Permeation properties of a compound, its distribution in an organism, and its interactions with body constituents are known to be largely dependent on the lipophilicity of the compound. Among various molecular parameters which can be considered as quantitative criteria of lipophilicity, partition coefficients P have been most widely used. In particular, the partition coefficient in the system octanol/buffer has become recognized as an operational definition of relative lipophilic character. Modern views on partition coefficients have been discussed in recent work[3–5].

The concept of partition coefficients has been extended by several authors to systems in which the homogeneous organic phase is replaced by heterogeneous biological or supramolecular phases like membranes or liposomes[6–10], or subcellular fractions[11]. In these cases the concentrations in the separated biological structures and their aqueous environment are determined. Due to methodological differences, comparison with partition coefficients obtained with bulk solvents becomes questionable.

Distribution of drugs in biological preparations can be determined with equilibrium dialysis techniques[12]. If the biological material in the buffer of one dialysis chamber is replaced by a bulk solvent, then the solvent/buffer partition coefficients of a drug can be determined. In this study basic and acidic model drugs were used to demonstrate the feasibility of the dialysis technique for the determination of partition coefficients. The major advantage is that this unifying technique provides direct comparisons between

Table 1. Partition coefficients (log P) of model drugs

		1-octanol	Olive oil	Arachis oil	Cyclohexane	Paraffin
Pentobarbital	log P[a]	2.0		0.8		
	log P[b]	2.20	1.00	0.68	−0.20	0.15
	% SD[c]	7	–	–	25	–
	n	15	2	2	7	2
Thiopental	log P[a]	2.9	1.9		0.5	
	log P[b]	2.87	1.87	1.44	0.62	0.53
	% SD[c]	22	–	–	4	–
	n	63	4	4	12	4
Imipramine	log P[a]	4.4				
	log P[b]	4.49			3.23	
	% SD[c]	50			17	
	n	15			6	
Chlorpromazine	log P[a]	5.3				
	log P[b,d]	5.03				
	% SD[c]	74				
	n	11				

True partition coefficients of non-ionized species. [a]Mean literature values[2]. [b]This study. [c]SD in percent of numerical (not log) mean value. [d]Determined after removal of photodegradation products[15].

Table 2. Distribution coefficients (log P′) at pH 7.4. Comparison of organic solvents and biological preparations

Solvent or preparation	Pentobarbital (3–5)	Thiopental (4–12)	Imipramine (3–4)
1-octanol	1.98	2.10	2.31
Olive oil	0.91	1.61	2.10
Arachis oil	0.58	1.19	1.85
Cyclohexane	−0.82	0.52	2.43
Paraffin	0.03	0.24	2.04
Liver homogenate	0.18	1.05	2.49
Brain homogenate	0.82	0.71	2.05
Muscle homogenate	0.65	0.76	1.72
Adipose tissue homogenate	0.81	1.58	2.24
Plasma	0.37	0.85	0.90
Lecithin liposomes	1.57	1.75	3.15

Tissues and plasma from rats. In brackets number of experiments. Drug concentration 200 nmoles/ml.

partition into organic phases and distribution into heterogeneous biological phases like membrane preparations, subcellular fractions, or tissue homogenates.

Materials and methods. ^{14}C-labeled thiopental, imipramine, and chlorpromazine were purchased from ICN Pharmaceuticals, Irvine, CA, USA; The Radiochemical Centre, Amersham, UK; and Applied Science Labs, State College, PA, USA; respectively. Pentobarbital was determined by direct spectrophotometry in the aqueous phase[13]. Paraffin subliquidum, olive oil, and hydrogenated arachis oil, as specified by Pharmacopeia Helvetica VI were obtained from Siegfried Inc., Zofingen, Switzerland. All other solvents and chemicals used were of analytical grade. Phosphate buffers 0.05 M, I = 0.2, of pH 4.5–11.5 were used throughout. Rat lean tissue homogenates were prepared with a glass homogenizer. Adipose tissue (perirenal) homogenate was obtained by sonication of the minced tissue. Suspensions of multilamellar lecithin liposomes were prepared by swelling egg yolk lecithin (Merck, Darmstadt, FRG) overnight with a 5-fold amount of buffer pH 7.4 and subsequent sonication by 6 bursts of 30 sec with intervals of 30 sec under ice cooling using a MSE 150 W sonicator.

For partition and distribution experiments 1.0 ml buffer was introduced into each chamber of a Dianorm dialysis apparatus[14] and an additional 0.2 ml organic solvent to the left chamber. The chambers were separated by a Visking regenerated cellulose membrane (mol.wt cut-off 12,000). 400 nmoles of the unionized form of the drug were dissolved in the organic phase (0.2 ml) or the same amount of the ionized form in the buffer phase. Partition equilibrium was reached by gentle rotation (20 rpm) of the double chambers for 3 h at 37 °C. After centrifugation of the contents of the left chamber the drug concentrations were determined in both the organic and aqueous phases. Total recoveries amounted to 92–98%. The organic phases did not visibly diffuse across the dialysis membrane separating the 2 chambers.

The pH-dependent distribution coefficient was calculated as $P' = c_{org}/c_{aq}$. The (true) partition coefficient P of a nonionized acid was determined both by using the asymptote of the pH-partition curve and the formula $P = P'(1 + 10^{pH-pK}a)$ (the exponent being pK_a-pH for bases). Distribution of drugs between buffer and heterogeneous biological phases such as tissue homogenates was determined by using the equilibrium dialysis device and simulating the conditions used in the partition experiments. 1 ml of buffer pH 7.4 or 7.5 was introduced into the right dialysis chamber as before. To the left chamber were added 1.2 ml buffer containing homogenate prepared from 0.2 g of the wet wt tissue, liposomes prepared from 0.2 g lecithin, or 0.2 ml of plasma. All other conditions were also as indicated above. At distribution equilibrium the drug was determined in the chamber containing buffer only. The experimental set-ups are shown in figure 1.

Results. Standard conditions were based on the results of

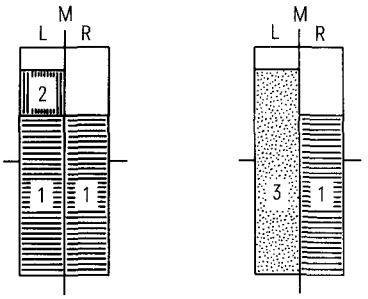

Figure 1. Experimental setup for the determination of distribution coefficients with organic solvents (left) and with biological preparations (right) using dialysis chambers. L, left chamber; M, dialysis membrane; R, right chamber; 1, buffer; 2, organic solvent; 3, suspension of biological material.

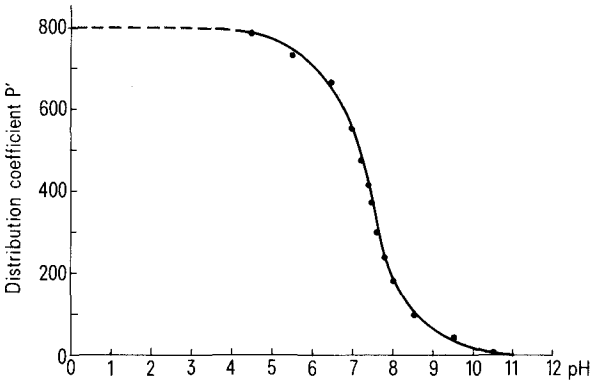

Figure 2. pH-partition profile of thiopental in 1-octanol/buffer. Thiopental acid dissolved in organic phase.

experiments with variable individual parameters in the system 1-octanol/buffer pH 7.4. They showed that distribution coefficients were not dependent on drug concentration (50–800 μM), volume of organic phase (0.1–0.4 ml), or equilibration time (3–48 h). The P′-values were not significantly different if the conventional shake-flask method was used instead of the dialysis method. All the following results were obtained using the standard experimental conditions as indicated above. The pH-partition profile of thiopental is shown on figure 2. The curve discloses a true partition coefficient P of around 800. Calculation of P from the P′-values yields a mean P of 748 ± 22. Thus, the results of the 2 methods for the determination of P differed by only 6%. With both 1-octanol and cyclohexane the inflection point of the pH-partition curve, i.e., the pK_a of thiopental, was at pH 7.5.

Table 1 displays the true partition coefficients P for thiopental, pentobarbital, imipramine, and chlorpromazine with 1-octanol, cyclohexane, paraffin, olive oil, and arachis oil.

Table 2 is a first attempt at comparing distribution coefficients P′ (pH 7.4) obtained with the 5 organic solvents previously used, with those obtained in corresponding experiments where tissue homogenates, plasma, and lecithin liposomes replaced the solvents.

Discussion. The true solvent partition coefficients (P) obtained, as well as the distribution coefficients (P′) for given pH-values were compared with those in the data collection of Hansch and Leo[4] and in the recent original literature. For thiopental and pentobarbital[16], for imipramine[17,18] and for chlorpromazine[15,17] there was good agreement with the literature values given.

Determination of the pH-partition profile of a drug yields its pK_a-value in addition to P and P′-values[5,15,19]. The pK_a-values determined for thiopental, pentobarbital, chlorpromazine, and imipramine were 7.5, 8.0, 9–9.5, and 9–10, respectively, and agree with the literature values. The values were identical regardless of whether they were derived from the octanol- or the cyclohexane curves. In the case of imipramine our results confirm the pK_a-values of 9.4–9.6 reported by Green[20], Seiler[21], and Thoma and Albert[22] whereas the values 8.6[23] and 8.0[24] seem to be erroneous. The calculated P-value of imipramine was based on $pK_a = 9.5$.

Whereas the SD of P'_{oct} were 22 and 7% of the means for thiopental and pentobarbital, respectively, they were about 50% for imipramine and even more for chlorpromazine. This is due to the fact that with the latter 2 drugs the concentration in the aqueous phase is only 1/100,000 of that in the organic phase and thus is close to the limit of sensitivity of the analytical method used. Still, an experimental error of 50% under these conditions is not unacceptable in the case of P_{oct} which spans a scale of at least 10 orders of magnitude.

The comparison of our model compounds based on P- or P′-values, with several organic phases, and starting from ionized or nonionized species, discloses an unequivocal increase in lipophilicity in the order pentobarbital < thiopental < imipramine < chlorpromazine. This sequence is based on determinations under identical conditions. The fact that the 2 basic drugs are more lipophilic than thiopental is important in view of the totally different distribution patterns of these drugs, particularly in adipose tissue[12,13].

Distribution coefficients of the model drugs with biological preparations can now be directly compared with P′-values obtained with organic bulk solvents (table 2). P'_{oct} was much greater than the corresponding cyclohexane value with the barbiturates, but not with imipramine. In contrast to octanol, partition coefficients with hydrocarbons are also related to H-bond breaking and desolvation[5]. Paraffin as another hydrocarbon gave P′-values comparable to cyclohexane with each of the model drugs. Similarly, the results with olive oil and hydrogenated arachis oil were in the same range.

The distribution coefficients of the model drugs with homogenates of liver, brain, muscle, and adipose tissue were within the ranges of octanol, hydrocarbons, or triglyceride phases. The likely reason for the differences observed is that biological phases also interact with drugs by forces other than mere hydrophobic interaction. For instance, it was reported that the interaction of chlorphentermin with subcellular fractions had a specific, saturable component as well as a nonspecific partitioning component[11]. P′ of plasma is likely to represent the former type of interaction. It is noteworthy that with each drug the P′-values with adipose tissue homogenate and with olive oil are almost identical. However, these values are considerably greater for imipramine, which hardly enters adipose tissues in vivo, in contrast to thiopental. With lecithin the high P′-values of imipramine (and chlorpromazine) seem to reflect the phospholipid binding of these drugs[25,26]. Reported lecithin values for barbiturates[9,27] and chlorpromazine[8] agree with our results. The distribution coefficients obtained with biological preparations may prove valuable when compared with standardized distribution studies in vivo. Experiments along these lines are now in progress.

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Announcements

Correction

T. Masukawa, J. Goto and H. Iwata: Impaired metabolism of arachidonate in selenium-deficient animals, Experientia *39* (1983) 405.

In table 3, page 405, there is a misprint. Table 3 should correctly read:

Effect of dietary selenium on the generation of PGI_2-like substance in rat aorta

Diet	% Inhibition of ADP-induced platelet aggregation
Se(−)	16.45 ± 3.28 (8)
Se(+)	31.93 ± 6.21* (8)

Each value represents mean ± SEM of 8 experiments. Significantly different from selenium-deficient group (*$p < 0.05$).

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