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NON-STEROID ANTI-INFLAMMATORY DRUGS: INFLUENCE OF EXTRA-CELLULAR pH ON BIODISTRIBUTION AND PHARMACOLOGICAL EFFECTS

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Abstract—Former observations on the biodistribution of (acidic) non-steroid anti-inflammatory drugs (NSAID) prompted us to investigate whether NSAID influence cell functions to a greater extent when acting under acidic extracellular pH conditions *in vitro*, and, if so, whether their enhanced effects might be due to an altered biodistribution of NSAID between extracellular space and cell interior under these conditions. The following experiments were done: Firstly, the effect of NSAID on phagocytosis and antimicrobial activity of human granulocytes was measured under normal (pH 7.4) and acidic (pH 6.8) conditions. NSAID were found to be most effective under low extracellular pH conditions whilst, alkaline drugs, serving as controls, were more effective at pH 7.4. Secondly, the influence of pH changes on the distribution of an acidic and an alkaline pyrazolone derivative between buffer and erythrocytes (or erythrocyte components) was measured. Lowering of extracellular pH caused an 80 per cent increase of the cellular concentration of the acidic NSAID while it (slightly) decreased the cellular concentration of the alkaline derivative. Ion trapping together with increased binding to cellular components was found responsible for this effect. These experiments show that the acidic environment of cells may cause accumulation of (acidic) NSAID within cells and thus enhance inhibition of various cell functions. The observations reported further support our concept that biodistribution is an important factor for the effects and side effects of NSAID.

When Vane discovered that non-steroid anti-inflammatory drugs (NSAID) almost uniformly have the capacity to interfere with prostaglandin synthesis [1] it was hoped that the mode of action of these important drugs was finally understood. Today these hopes appear premature because there is now ample evidence that prostaglandin synthesis inhibition alone is not sufficient to explain the pharmacological effects of NSAID [2]. Especially, one question remains unanswered, namely, why only *acidic* NSAID have found broad acceptance in the clinic, despite the fact that there are many alkaline analogues which are potent anti-inflammatory compounds in various *in vitro* models of inflammation, and do not show gastric irritancy and kidney damage, the common side effects of the acidic NSAID [3]. This question may be answered by our recent observation that only acidic NSAID accumulate in inflamed tissue but also the stomach wall and the kidney tubules, i.e. the main sites of therapeutic effects and side effects of these drugs. From this observation we speculated that selective accumulation of acidic NSAID in certain compartments firstly, is intrinsically connected with the pharmacological effects in these compartments and secondly, is caused by the fact that in these compartments cells are surrounded by, or at least bordering on, acidic extracellular milieu in contrast to most other cells in the body which are surrounded by slightly alkaline fluids. We feel that this concept might well help to understand why only weak acids are useful anti-inflammatory drugs but also why these drugs comprise a relatively uniform profile of side effects. However, we believe that this concept needs further experimental proof. Most importantly, it needs to be shown, that acidic NSAID interfere with cell functions more effectively under acidic extracellular conditions. In addition, it is necessary to find out whether our former observations of drug accumulation in, e.g. inflamed tissue is due to an increased drug concentration within the cell. To investigate these questions, functional studies using human polymorphonuclear granulocytes (PMN) and distribution studies with human erythrocytes (ER) were performed *in vitro*.

MATERIALS AND METHODS

Functional studies. Human polymorphonuclear leukocytes (PMN) served as examples of cells functioning in inflamed tissue. Using an *in vitro* test system we analyzed the effect of pH on the action of different drugs upon phagocytosis and antimicrobial activity of PMN. All the drugs tested had some antiinflammatory and/or analgetic potency but some were acids and some were bases.

In a previous paper [4] we have described a simple but sensitive method to evaluate phagocytosis based on three known facts: (a) PMN adhere to glass, (b) vital PMN accumulate dyes, e.g. Methylene Blue (MB) in phagolysosomes, and (c) dead Candida yeasts stain blue with the same dye. Starting from these facts we found that MB could be used to detect by microscopic observation whether or not C. albicans cells in the phagocytic vacuole of vital PMN were dead. For the test procedure, monolayers of PMN were

obtained from 3 drops of capillary blood (ca 0.1 ml) dripped directly on a cover slip forming the bottom of a hole (11.3 mm diameter) drilled in a 1-cm thick piece of perspex. The top of the hole was sealed with a piece of adhesive tape. The so formed moist chamber was stored for 1 hr at 37°. Thereafter, the tape was removed, the clotted blood was discarded, and the remaining monolayers of leukocytes were gently rinsed with warm (37°) saline. They were then ready for experiments assessing the effect of drugs on phagocytosis and intracellular killing of ingested microorganisms.

PMN. The PMN investigated were obtained from a panel of twenty healthy male volunteers. The same donors served for the evaluation of drug effects on PMN functions under different pH conditions.

Candida. Sabouraud broth was inoculated with C. albicans from a stock culture kept on Sabouraud agar. After 3–7 days of incubation at 33°, C. albicans in yeast phase was harvested by centrifugation of the medium at 250 *g* for 10 min. The pellet was washed twice and suspended in Dulbeccos phosphate buffered saline of the desired pH. The C. albicans cells were counted in a hemocytometer, and the suspension was adjusted to 2×10^6 C. albicans cells/ml. The number of dead C. albicans in this suspension was determined by counting the blue cells under the microscope after adding MB (final concentration 2×10^{-4} M). Only specimens containing less than 1.0 per cent dead C. albicans were used.

Serum. Serum was prepared from blood of healthy donors and frozen in 1 ml portions at −70°. It was thawed only immediately before use, centrifuged and brought to the desired pH by slow (1 hr) addition of 0.1 N HCl.

Drugs and chemicals. Antipyrine, procainamide HCl and phenobarbital were U.S.P. grade. Phenylbutazone was a gift of Ciba–Geigy AG, Basel, Switzerland. All other chemicals used were of analytical grade, obtained from usual commercial sources. The drugs were dissolved in the buffers used for the experiments in ten times the final concentration.

Test procedure. To the PMN monolayers were added 120 μl of C. albicans suspension, 15 μl of serum and 15 μl of drug solution or buffer, all being at 37°.* The moist chamber was sealed with a membrane permeable to atmospheric gases and incubated on a rotating disk (Multi purpose rotator, model 150 V, Scientific Industries Inc., Springfield, MA; 10 r.p.m.) at 37°. Two minutes before the end of the incubation period 50 μl of Hank's balanced salt solution with MB (8×10^{-4} M) was added. At the end of the incubation period the chamber was removed and turned upside down, and the monolayer was observed under the microscope through the cover slip forming the bottom of the chamber. To quantitate the phagocytosis, the C. albicans inside 100 PMN were counted, and the average number of C. albicans per 100 PMN was calculated. The killing of C. albicans was assessed by counting the number of C. albicans stained blue inside 100 PMN. The count was corrected with the percentage of C. albicans found dead in controls containing C. albicans and serum only. The killing was then expressed as the number of killed C. albicans per 100 PMN or as the ratio of killed C. albicans to phagocytized C. albicans. Experiments were always done in duplicate, and the mean was taken as one value.

Distribution studies. To evaluate the effect of a slightly acidic extracellular pH on the distribution of an acidic (phenylbutazone) and a weekly alkaline pyrazolone derivative (antipyrine) between a buffer phase and cells or cell components the following experiments were done: Firstly, we measured the concentration of these drugs in erythrocytes (ER) suspended in a buffer system of different pH. Secondly, we analysed the effect of pH-variations on the distribution of these drugs between buffer and ER or lysed ER using equilibrium dialysis.

Drugs. [^{14}C]Phenylbutazone was supplied by Ciba–Geigy AG, Basel, Switzerland, [^{14}C]antipyrine was obtained from the "Radiochemical Centre, Amersham". The drugs were "diluted" with appropriate amounts of cold drug (if necessary) and dissolved in isotonic phosphate buffer to give the final concentrations of 5×10^{-6} M. Purity and stability of these solutions was monitored by u.v. spectrophotometry or thin layer chromatography.

Buffers. All experiments were done using phosphate buffered saline (5×10^{-3} M sodium phosphate in 1.5×10^{-1} M sodium chloride) of different pH. In this buffer ER did not show hemolysis or morphological decay within 24 hr at the pH values employed.

Erythrocytes (ER). Whole blood was obtained from healthy donors. Cells (approx 97 per cent ER) were prepared by washing whole blood cells three times in phosphate buffered saline of the desired pH. ER were microscopically checked for morphological integrity. ER showing morphological changes or hemolysis were discarded. For some experiments samples or ER suspensions were lysed by abrupt freezing in liquid nitrogen followed by thawing before employing equilibrium dialysis.

Firstly, drug distribution between buffer and ER was measured as follows. ER (10 per cent v/v) were added to the buffer of either pH 6.8 or 7.4 (containing either one of the pyrazolone derivatives). This suspension was gently shaken and the pH kept constant for 1 hr using an automatic titrator and NaOH 0.01 N or HCl 0.01 N. Then the suspension was centrifuged (1600 *g* for 5 min), the supernatant (upper 75 per cent of the total volume) removed and the sediment (plus the remaining supernatant) lysed with Triton X-100 (10 μl of a 10 per cent solution was added to 1 ml sediment). (Longer incubation times did not change the distribution of the drugs used measurably.) Samples were then handled as described in the next section. The experiments were done in parallel for both pH values, 6.8 and 7.4, using five test tubes for each condition. They were repeated four times.

Secondly, equilibrium dialysis was employed to establish to which extent changes in partitioning of the drugs were due to ion-trapping in the cell interior and/or changes in the binding to ER-components. In these experiments ER or lysed ER in buffer were dialysed against buffer containing ^{14}C-labelled drugs in double chambers separated by dialysis membranes for

* In former experiments [4] instead of phosphate buffered saline, Hank's balanced salt solution was used. Without drugs, both media are equally well tolerated by PMN as judged from their functional competence.

22 hr at room temperature. The chambers were sealed with a membrane permeable to atmospheric gases. At 22 hr equilibrium was reached in all cases, ER and ER-components were equilibrated with the buffer used before the experiments were started. The dialysis membranes were also equilibrated with buffer containing "cold" drug. After 22 hr of dialysis intact ER were checked for integrity and the content of all chambers for bacterial growth. Experiments showing bacterial growth were rejected. Again, the experiments were done in parallel for both pH values, 6.8 and 7.4, using five test tubes for each condition. They were repeated four times.

Evaluation. Samples of the supernatants and the lysed sediments as well as from both dialysis chambers were counted in a Packard Tri-carb Liquid Scintillation Spectrometer and the results were corrected for background and quenching using an internal standard. In addition, samples obtained from the same sources were assayed for protein content to assure that lysis of ER or leaks in the dialysis membrane had not occurred and to allow for the calculation of the hematocrit. From these data the drug concentration in the ER and buffer compartments was calculated. When lysed ER or ER-components were used the drug concentrations were calculated for the volume of ER before lysis or containing the same amount of ER-components as were employed in the experiment. From these results the means and standard deviations of the partition coefficient (P) were calculated for each experimental condition. In addition the influence of varying the pH on the drug distribution was expressed as

$$Q + S.D. = \frac{P_{6.8}}{P_{7.4}} \pm \frac{P_{6.8}}{P_{7.4}} \times \left(\frac{S.D.P_{6.8}}{P_{6.8}} + \frac{S.D.P_{7.4}}{P_{7.4}} \right)$$

Similarly the influence of pH on the drug effects on PMN function was calculated.

RESULTS

I Leukocyte (PMN) function

(1) *The effect of lowering the extracellular pH.* PMN are quite capable of performing their antimicrobial activity in an acidic environment (Table 1). Neither phagocytosis nor intracellular killing were significantly altered under these conditions. However, there appears to be a slight reduction of both functions which has to be taken into account when the drug effects are assessed.

(2) *The effect of drugs.* Alkaline and acidic drugs proved to be inhibitors of PMN functions *in vitro* (Tables 1 and 2). However, both types of drugs had to be employed in relatively high concentrations to cause a significant inhibition of PMN functions. Also, both types of drugs influenced phagocytosis as well as intracellular killing and thus the drug effect was most pronounced when the number of C. albicans killed per 100 PMN was measured (Table 1). Therefore this parameter was chosen for expressing the effect of a variety of drugs and drug concentrations under different pH conditions (Table 2).

(3) *The effect of pH and drugs.* In Table 2 the effect of two acidic drugs, a weak, almost neutral base and two strong bases on PMN function under acidic and alkaline pH conditions are given. Although the effect of pH variation shall not be overemphasized it is clear that the two acidic drugs are more effective in an acidic environment whilst the alkaline drugs are much more effective inhibitors of leukocyte function in an alkaline milieu. For example, phenylbutazone (10^{-4} M) reduced the number of killed C. albicans per 100 PMN to 17 per cent at pH 6.8 whilst at pH 7.4 still 41 per cent were killed. This pH effect was significant. On the other hand, procainamide in the same concentration reduced the rate of killing only to 50 per cent at pH 6.8. while at pH 7.4 the killing of C. albicans was reduced to 22 per cent. Again this effect, going in the other direction, was significant.

II Drug distribution

PMN cannot easily be kept in suspension *in vitro*. They aggregate, ingest and destroy each other. Hence the effect of pH on drug distribution could not be studied using these cells. Human erythrocytes were used instead. For these studies we concentrated on two pyrazolone derivatives, namely, phenylbutazone and antipyrine which were available in labelled form and had shown a different activity profile in the functional studies, i.e. the acidic phenylbutazone ($pK_a = 4.8$) was more effective in inhibiting PMN functions at acidic pH whilst the weak base antipyrine ($pK_a = 1.4$) was almost equally effective (or ineffective) at both pH conditions.

(1) The pH effect on drug distribution between erythrocytes and buffer. In Table 3 the results of drug partitioning studies using whole erythrocytes are given. Whilst antipyrine distributes almost equally between buffer and cells, phenylbutazone is found in higher concentrations in the cell fraction, both under acidic and alkaline conditions. Moreover, lowering the pH from 7.4 to 6.8 further increased the concentration of phenylbutazone in the cell fraction by about 80 per cent. On the other hand variation of the pH did not cause a measurable change in the distribution of antipyrine. Similar results were obtained by equilibrium dialysis-experiments.

(2) The pH effect on drug distribution between erythrocyte lysate and buffer. To find out whether ion trapping contributes to the accumulation of phenylbutazone in erythrocytes in an acidic environment erythrocytes were lysed and equilibrium dialysis was performed with the whole lysate. The results can be compared with the data obtained with intact ER. Table 3 shows that there is a clear cut reduction of drug accumulation when the erythrocytes were lysed in comparison to intact. Nevertheless under these conditions there still was a significant pH effect, i.e. lowering of the pH caused an accumulation of phenylbutazone in the chamber containing the lysed erythrocytes. This indicates an increased binding to the components of the disintegrated erythrocytes. Again, no such effect could be detected with antipyrine.

DISCUSSION

(I) *PMN as target of NSAID*

In our experiments, PMN were taken as example of cells which accumulate and function in inflamed tissue. These cells are *in vivo* exposed to the slightly

Table 1. Inhibition of phagocytosis and intracellular killing of C. albicans by human PMN: Effects of an acidic and an alkaline drug under different pH conditions

Drugs	(M)	N	pH	C. albicans ingested per 100 PMN Mean ± S.D.	%	P*	C. albicans killed per 100 PMN Mean ± S.D.	%	P*	C. albicans killed per 100 C. albicans ingested Mean ± S.D.	%	P*
Controls		14	6.8	225 ± 28.6	—	—	24 ± 4.6	—	—	11 ± 2.5	—	—
		16	7.4	242 ± 36.8	—	—	27 ± 5.1	—	—	11 ± 2.8	—	—
Phenylbutazone	10^{-4}	10	6.8	117 ± 37.2	52	<0.01	4 ± 2.1	17	<0.01	3 ± 2.5	27	<0.01
		8	7.4	149 ± 28.1	62	<0.01	11 ± 3.2	40	<0.01	7 ± 2.2	64	<0.01
Procainamide	10^{-4}	8	6.8	138 ± 47.3	61	<0.01	12 ± 4.3	50	<0.01	9 ± 3.1	82	N.S.
		11	7.4	105 ± 35.6	43	<0.01	6 ± 3.1	22	<0.01	6 ± 2.8	55	<0.01

N: Number of experiments.
%: % of controls.
*: As compared with controls (Student's *t*-test).
N.S.: $P > 0.05$.

Table 2. Inhibition of PMN functions by drugs of different pK_a values under different pH conditions

Drug	pK_a	(M)	C. albicans killed per 100 PMN at pH 6.8 N	Mean ± S.D.	%	P*	at pH 7.4 N	Mean ± S.D.	%	P	Effect of lowering of pH Q	P^+
Controls			14	24 ± 4.6	—	—	16	27 ± 5.1	—	—	0.89 ± 0.34	N.S.
Phenylbutazone	4.8 acid	10^{-5}	8	11 ± 3.7	46	<0.01	8	18 ± 3.6	67	<0.01	0.61 ± 0.33	<0.01
		10^{-4}	10	4 ± 2.1	17	<0.01	9	11 ± 3.2	41	<0.01	0.36 ± 0.30	<0.01
Salicylic acid	3.0 acid	10^{-4}	6	16 ± 5.4	67	<0.01	6	20 ± 4.2	80	N.S.	0.80 ± 0.48	N.S.
		10^{-3}	9	5 ± 2.1	21	<0.01	8	9 ± 2.3	36	0.01	0.56 ± 0.38	<0.01
Antipyrine	1.4 base	10^{-5}	7	23 ± 4.4	96	N.S.	8	26 ± 5.3	96	N.S.	0.89 ± 0.35	N.S.
		10^{-4}	11	21 ± 6.1	88	N.S.	10	22 ± 4.8	81	<0.05	0.95 ± 0.48	N.S.
Benzydlamine	~9 base	10^{-6}	9	17 ± 3.8	71	<0.01	9	16 ± 5.1	59	<0.01	1.06 ± 0.58	N.S.
		10^{-5}	9	13 ± 2.6	54	<0.01	10	10 ± 3.3	27	<0.01	1.30 ± 0.69	N.S.
Procainamide	11 base	10^{-5}	11	15 ± 5.6	63	<0.01	8	13 ± 2.5	33	<0.01	1.15 ± 0.65	N.S.
		10^{-4}	7	12 ± 4.3	50	<0.01	11	6 ± 3.1	22	<0.01	2.00 ± 1.75	<0.01

N: Number of experiments.
%: % of controls.
*: As compared with controls (Student's *t* test).
N.S.: $P > 0.05$.
Q: Fractional change due to lowering of pH (mean ± S.D. at pH 6.8 divided by mean ± S.D. at pH 7.4; for calculation see Methods).
+: As compared with experimental group at other pH.

Table 3. Effect of pH changes on the distribution of antipyrine and phenylbutazone between erythrocytes or erythrocyte components and buffer

		Phenylbutazone			Antipyrine		
		pH 6.8 Mean* ± S.D.	pH 7.4 Mean ± S.D.	Differ. P†	pH 6.8 Mean ± S.D.	pH 7.4 Mean ± S.D.	Differ. P
ER in Suspension	P‡	8.22 ± 0.98	4.56 ± 0.63	<0.01	0.94 ± 0.15	0.95 ± 0.17	N.S.
	Q§		1.80 ± 0.46			0.99 ± 0.33	
ER Equil. Dial.	P	7.27 ± 0.99	4.39 ± 0.19	<0.01	0.89 ± 0.10	0.88 ± 0.17	N.S.
	Q		1.66 ± 0.27			1.0 ± 0.31	
ER Lysed	P	5.73 ± 0.36	3.96 ± 0.19	<0.01	0.94 ± 0.26	0.95 ± 0.22	N.S.
	Q		1.45 ± 0.14			0.99 ± 0.50	

* Means and S.D. from 5 experiments done in parallel, similar results were obtained when the experiments were repeated (4 times).

† As compared with the results obtained at the other pH (Student's *t*-test).

‡ *P* = partition coefficient: drug concentration in erythrocytes divided by concentration in buffer. Partitioning measured as described as follows: erythrocytes (ER) were exposed to buffer (BU) containing drug for 2 hr and then separated by centrifugation (see Methods).

§ Fractional change due to lowering of pH ($P_{6.8}$ divided by $P_{7.4}$, for calculations see Methods).

N.S.: $P > 0.05$.

acidic conditions in inflamed tissue [5] and they are, if NSAID are given, exposed to comparatively high concentrations of these drugs in the inflamed tissue [6]. According to the concept of non-ionic diffusion even by lowering the extracellular pH slightly, considerable ion trapping should occur as shown in Table 4. Our *in vitro* results support this assumption in part. Phenylbutazone is more effective in impairing PMN function in an acidic environment. However, high concentrations, which can hardly be achieved *in vivo* are necessary to achieve this effect. These observations are in line with previous observations which have shown that PMN are quite resistant to effects of NSAID *in vitro* [7–9] and *in vivo* [10] and they explain why these drugs apparently do not promote bacterial growth in bacterial inflammations *in vivo* [11]. PMN obviously do not comprise a prime target of NSAID. However, there is good indication that other cells of the inflamed tissue as, e.g. neuronal cells, which do not lend themselves for functional studies, may be more likely targets of these drugs. They clearly have local anaesthetic effects in contrast to narcotic analgesics [12] which act within the CNS. One may now speculate why PMN are able to function as well in inflamed tissue under the influence of NSAID. One explanation is that these mobile blood cells, which have to function wherever and whenever tissue damage occurs in the body, have especially active transmembrane transport mechanisms for organic acids or bases which allow for a certain degree of control of the internal milieu. Indications that this may be so are coming from experiments using slime molds which are completely resistant to these drugs even at a pH of 4. Nevertheless with unphysiological concentrations of NSAID even in PMN, pH dependent inhibition of cell functions becomes measurable. This effect cannot be explained by a decrease in binding of, e.g. phenylbutazone to plasma proteins since this drug was found to be 99 per cent bound to plasma proteins at pH 7.4 as well as at 6.8 [21].

(II) *PMN and erythrocytes as models of cells of inflamed tissue*

Barker and Levitan could show that acidic NSAID might exert their local anaesthetic effects by interfering with cation-permeability of neuronal membranes [13] thus causing hyperpolarisation and reduced irritability of these substrates. Also, they produced evidence that the salicylates were especially active in in-

Table 4. Influence of variation of the extracellular pH on the calculated concentration of phenylbutazone in the extracellular and intracellular space

	Normal tissue		Inflamed tissue	
	Extracellular space	Intracellular space	Extracellular space	Intracellular space
pH	7.4	7.0	6.8	7.0
Concentration PH	0.2	0.2	1.0	1.0
Concentration P^-	99.8	39.8	99.0	158.0
Concentration P_{tot}	100	40	100	159

The calculation is done making the following assumptions: (a) The cell membrane is impermeable to phenylbutazone ions (P^-) but freely permeable for protonated phenylbutazone (PH). Hence the concentration of PH is equal in the extra- and intracellular space while the concentration of P^- varies according to different degree of ionization of phenylbutazone (P: $pK_a = 4.8$) in the two compartments. (b) The total concentration of phenylbutazone (P_{tot}) is assumed to be equal in the extracellular space in inflamed and not inflamed tissue ($P_{tot} = 100$). If these assumptions are correct, lowering of the extracellular pH should increase the intracellular concentration of P almost four times. This calculation takes into account only the amount of P not bound to proteins.

hibiting this process when the environmental pH of the neurons was lowered [14]. However, an extension of these results on the conditions in inflammation in man was not without risk because comparable results could not be obtained in man until now [15]. We could recently show that many NSAID tested accumulate in inflamed tissue but also in two other compartments where these drugs have effects and where cells border acidic fluids, namely the kidney tubules and the stomach wall [2]. We believe that the results reported in this paper add to the former observations of Barker and Levitan and from our group.

Firstly, using human cells, we could show that NSAID can inhibit cell functions and they do this in a pH dependent manner. Already a slight lowering of the pH as it has been observed in inflamed tissue [5] caused a considerable increase of the concentration of acidic NSAID in the cell interior. This may explain why extracellular pH, pK_a and lipophilicity of these drugs are so important for their potency *in vivo* and *in vitro* [13, 16–18]. It also may add to the understanding of the well known observation that local anaesthetics which are bases as, e.g. procainamide lose potency in inflamed tissue. Our results together with Barker and Levitan's observations further indicate that inhibition of cell functions by acidic NSAID may explain their local anaesthetic action in inflamed tissue which is not seen with narcotic analgesics [12].

Secondly, our results also indicate that the fairly selective accumulation of acidic NSAID in certain body compartments having acidic extracellular pH conditions may in part be due to increased concentrations of these drugs in the cell, i.e. where pharmacodynamic actions take place. This is important because high concentrations in the extracellular space are probably comparatively irrelevant events. Our observations are in line with previous findings showing increased toxicity of weak acids like salicylates and barbiturates [19, 20] going along with general acidosis and drug disappearance from the plasma compartment *in vivo*.

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