NEUROTOXICITY OF X-RAY CONTRAST MEDIA*

RELATION TO LIPID SOLUBILITY AND BLOOD-BRAIN BARRIER PERMEABILITY

By STANLEY I. RAPOPORT, M.D.,† *and* HERBERT LEVITAN, PH.D.‡
BETHESDA AND COLLEGE PARK, MARYLAND

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X-RAY contrast media that are employed in cerebral and spinal cord angiography occasionally produce neurologic complications that are manifestations of neurotoxicity. The experimental approach to neurotoxicity is confused, however, because multiple factors come into play and neurotoxic endpoints have not been well defined.[1] For example, hemodynamic responses to contrast media and breakdown of the blood-brain barrier have been used as endpoints,[17,37] but they are not necessarily related to the central nervous system involvement that represents neurotoxicity.[2,12,17,26,33,34,36]

Solutions of currently used x-ray contrast media (salts of triiodobenzoic acid derivatives) are as concentrated as 1.45 osmolal and can open the blood-brain barrier at equal osmotic concentrations of about 1.2 osmolal, when perfused into the internal carotid artery of rabbits.[34] They probably widen tight junctions between cerebrovascular endothelial cells by shrinking the cells osmotically. The continuous cerebrovascular endothelium constitutes the blood-brain barrier to passive entry of solutes into the brain and is permselective for lipid soluble solutes.[5,19,29,35]

Because they open the barrier at equal osmolalities, it is probable that differences in neurotoxicity among the x-ray contrast media are unrelated to osmotic barrier opening. Such differences may depend instead on specific physico-chemical properties that regulate media permeability through the barrier, such as lipid solubility and the aqueous dissociation constant.

Such a possibility has not been considered seriously before because salts of the triiodobenzoic acids are almost completely dissociated at physiologic pH.[13,15,24] Entry of organic electrolytes into the brain across the blood-brain barrier is thought to depend entirely on the concentration and lipid solubility of the undissociated lipid soluble form.[3,28]

The dissociated form of an organic electrolyte has, however, a finite lipid solubility.[10] Since the ratio between intravenous and intracisternal LD_{50} in mice is as high as 1,400:1 for some contrast media,[14] entry into the brain of even a small fraction of the arterial concentration may be sufficient to produce neurotoxicity.[20] This small entry may in part include the dissociated form. It was of interest therefore to estimate quantitatively the brain uptake of contrast media of both the dissociated and undissociated forms, and to see if neurotoxicity and systemic toxicity could be related to lipid solubility and concentration. An abstract of this paper has been published.[32]

METHOD AND RESULTS

RELATION OF NEUROTOXICITY OF X-RAY CONTRAST MEDIA TO LIPID SOLUBILITY

Recent reviews on x-ray contrast media that are derivatives of benzoic acid have concluded that they have distinct differences in their neurotoxicity.[1,12,13,36] The use of some has been discarded because of the high incidence of neurologic complications. In the United States, Conray, Hypaque and Isopaque remain in use. On the basis of these reviews, we rank ordered 8 contrast media anions with respect to neurotoxicity, as illustrated in Table 1. A rank of 3.5 for

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From the Laboratory of Neurophysiology,† National Institute of Mental Health, Bethesda, Maryland; and Department of Zoology,‡ University of Maryland, College Park, Maryland.

TABLE I

CORRELATION OF NEUROTOXICITY WITH OCTANOL/WATER PARTITION COEFFICIENT (P_{HA}) FOR 8 CONTRAST MEDIA*

Contrast Medium Anion	Commercial Name	Rank of Neurotoxicity	Log P_{HA}	Rank of Lipid Solubility
Iothalamate	Conray	1	1.96	2
Ioxithalamate	Vasobrix	2	1.30	1
Diatrizoate	Hypaque, Urografin	3.5	2.50	3
Metrizoate	Isopaque	3.5	2.60	4.5
Iodamide	Uromiro	5	2.60	4.5
Diprotrizoate	Miokon	6	3.50	7
Acetrizoate	Urokon	7	3.29	6
2,4,6-Triiodobenzoate	—	8	4.08	8

* Log P_{HA} values are taken from Table II. The rank correlation coefficient between neurotoxicity and lipid solubility is 0.94 ($P < 0.01$).

diatrizoate and metrizoate indicates that these 2 anions have the same neurotoxicity.

The lipid solubility of a compound can be defined as its tendency to be distributed selectively in a lipid rather than in an aqueous phase, and is conveniently quantified by its octanol/water partition coefficient.[22] The permeability of cell membranes and of the blood-brain barrier to a substance increases as the lipid solubility or partition coefficient of the substance increases.[4,5,27] As far as we know, partition coefficients have not been measured for the contrast media, and therefore we calculated octanol/water partition coefficients by using rules for their additive-constitutive properties.[6,8,10,22,23]

For a parent compound whose coefficient is P′ and for a derivative compound whose coefficient is P'_x, the additive constant π_x is defined as

$$\pi_x = \log P'_x - \log P'. \qquad (1)$$

For instance, if the parent compound is benzoic acid and the derivative compound is p-iodobenzoic acid,

$$\pi_{p-I} = \log P'_{p-I\ benzoic} - \log P'_{benzoic}$$

$$1.15 = 3.02 - 1.87.$$

Experimental log P′ values were obtained from tabulations by Leo *et al.*[22]

Table II illustrates how the octanol/water partition coefficients were calculated for the 8 contrast media anions in Table I. P_{HA} is the coefficient of the undissociated acid, HA. Adding iodide to benzoic acid increases P_{HA}, while adding the acetamide group decreases P_{HA}.

Comparison of the rank order of neurotoxicity with that of P_{HA} shows that the more neurotoxic agents are more lipid soluble (Table I), the rank correlation coefficient being 0.94 ($P < 0.01$). The exact rank of either neurotoxicity or lipid solubility may be unreliable because of the estimates that we make, but this significant correlation indicates their very close relation.

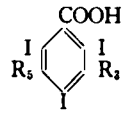
RELATION BETWEEN BRAIN UPTAKE AND LIPID SOLUBILITY

The correlation between octanol/water partition and clinical neurotoxicity suggests that a rate-limiting step regulating neurotoxicity is the passage of the contrast medium across cell membranes, presumably those of the blood-brain barrier.[5,19] The question arises as to whether brain uptake of contrast media is large enough to justify this suggestion, and is in the same quantitative order as neurotoxicity.

In the Appendix we calculate a partition parameter P which takes into account the relative concentrations of the undissociated form HA and dissociated form A^- of the contrast media acids, as well as their respective partition coefficients P_{HA} and P_A. We assume that net brain uptake of both forms of the organic acid will be a function

TABLE II

CALCULATION OF LOG P_{HA} FOR 2,4,6-TRIIODOBENZOIC ACID AND ITS DERIVATES*



Contrast Medium	Additions	Calculations	Log P_{HA}
2,4,6-Triiodobenzoate	R_3: −H R_5: −H	log $P_{benzoic}$ + π_{p-I} + $2\pi_{o-I}$ 1.87 +1.15+2(0.53)	4.08
Acetrizoate	R_3: −$NHCOCH_3$ R_5: −H	log P_{tri-I} + $\pi_{m-acetamido}$ 4.08 −0.79	3.29
Diatrizoate	R_3: −$NHCOCH_3$ R_5: −$NHCOCH_3$	log P_{tri-I} + $2\pi_{m-acetamido}$ 4.08 −1.58	2.50
Iothalamate	R_3: −$CONHCH_3$ R_5: −$NHCOCH_3$	log P_{tri-I} + $\pi_{m-acetamido}$ + $\pi_{m-CONH2}$ + $\pi_{CH3\ near\ NH}$ 4.08 −0.79 −1.49 +0.16	1.96
Metrizoate	R_3: −$N(CH_3)COCH_3$ R_5: −$NHCOCH_3$	log P_{tri-I} + $2\pi_{m-acetamido}$ + $\pi_{CH3\ on\ N}$ 4.08 −1.58 +0.1	2.60
Iodamide	R_3: $CH_2NHCOCH_3$ R_5: −$NHCOCH_3$	log P_{tri-I} + $2\pi_{m-acetamido}$ + $\pi_{CH3\ on\ N}$ 4.08 −1.58 +0.1	2.60
Ioxithalamate	R_3: $CONHCH_2CH_2OH$ R_5: −$NHCOCH_3$	log P_{tri-I} + $\pi_{m-acetamido}$ + $\pi_{m-CONH2CH3}$ + $\pi_{m-CH2OH}$ 4.08 −0.79 −1.33 −0.66	1.30
Diprotrizoate	R_3: $NHCOCH_2CH_3$ R_5: $NHCOCH_2CH_3$	log P_{tri-I} + $2\pi_{m-acetamido}$ + $2\pi_{CH3}$ 4.08 −1.58 +1.0	3.50

* Log P_{HA} values are composed additively of individual π_x values, as discussed in text. Log P_{tri-I} = log $P_{2,4,6-triiodobenzoic\ acid}$. Source of substituent constants is Leo *et al.*[22]

of the lumped partition parameter P, since blood-brain barrier permeability increases with increasing partition coefficient for solutes in general.[5]

Blood-brain barrier permeability and brain uptake of the benzoic acid derivates have not been measured, but recently a method has been developed by which brain uptake of 7 aliphatic monocarboxylic acids was determined.[25] A mixture of C^{14} tracer and of $H_2{}^3O$ (which easily crosses the barrier) was injected into the internal carotid artery of the rat and the brain was sampled 15 seconds later. The brain uptake index (BUI) is calculated as follows:

$$\text{Brain Uptake Index (BUI)} = \frac{\text{Brain } C^{14}/\text{Brain } H^3}{\text{Injectate } C^{14}/\text{Injectate } H^3} \quad (2)$$

Figure 1 shows that the BUI's of the aliphatic monocarboxylic acids increase with increasing values of log P, as proposed above. This supports the suggestion that the rate limiting step for passive brain uptake of these acids is their transfer across the lipoid membranes of the blood-brain barrier. BUI is not a linear function of blood-brain barrier permeability, however, because very high BUI's depend also on cerebral blood flow.

Log P in Figure 1 was calculated with the use of Equations 7 and 8 from experimental values of log P_{HA},[22] letting $pK_a = 4.8$[39] and blood pH = 7.4. P_A was taken as $10^{-4.1}P_{HA}$.[7,10] The experimental BUI's in Figure 1 exclude contributions made by carrier-mediated mechanisms to the brain uptake of some of the monocarboxylic acids.[25]

The BUI for the x-ray contrast media can be predicted from the BUI of the aliphatic monocarboxylic acids, assuming that the BUI of both classes depends on log P. Contrast media that are derivatives of benzoic acids have pK_a's closer to 3.0 than to 4.8.[15] In 50 per cent methanol-water, pK_a's range from 3.0 to 3.6,[24] and calculate out to be about 3.0 in aqueous solution,[16] (using a reaction constant $\rho = 1.085$).

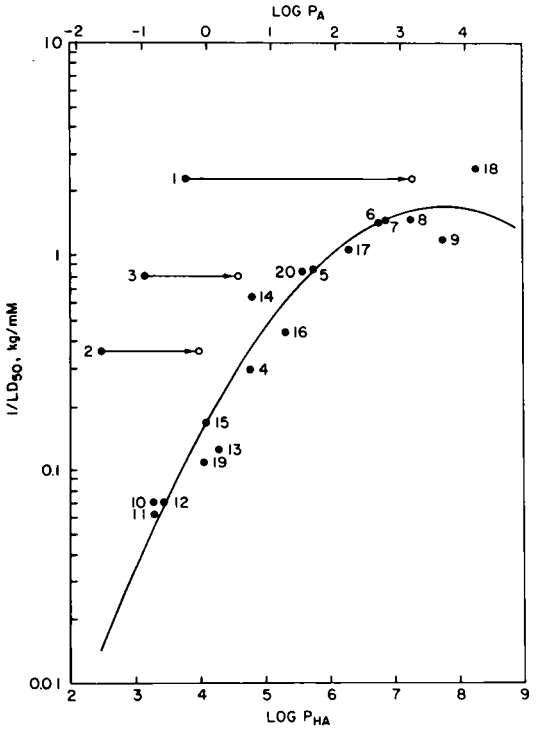


FIG. 1. *Relation of brain uptake of organic acids to octanol/water partition coefficients.* The brain uptake of an organic acid is quantified by the brain uptake index (BUI) which is the ratio of labeled acid to tritiated water in the brain relative to that injected into the internal carotid artery 15 seconds earlier[25] (see text). The experimentally determined uptakes of 7 aliphatic monocarboxylic acids (filled circles) are given on the ordinate, and the abscissa is log P as calculated from experimental log P_{HA},[22] $pK_a = 4.8$,[39] and pH = 7.4, as given by Equation 8. The experimentally determined BUI's exclude contributions made by carrier-mediated transport.[25] They are fit by the least squares curve given by the equation, log BUI = 4.459 + 0.824 log P − 1.161 (log P)^2. The open circles in the figure represent the predicted brain uptakes of the x-ray contrast media, where their log P_{HA} are given in Table 1, $pK_a = 3.0$, and log P = log P_{HA} − 3.92 (Equation 9).

By using Equation 7 in the Appendix, we were able to calculate log P for the contrast media at pH 7.4 from their log P_{HA} in Table 1, from $pK_a = 3.0$, and by letting $P_A = 10^{-4.1} P_{HA}$. The open circles in Figure 1 represent the predicted values of BUI for the x-ray contrast media acids, as based on observed BUI for the monocarboxylic acids. The lower pK_a's for the contrast media shift their effective partition parameter log P to the left of the values for the monocarboxylic acids, thereby decreasing their predicted brain uptake index.

As pointed out by Oldendorf,[25] a BUI of 2 per cent indicates that a substance is practically impermeant across the blood-brain barrier, while higher values show that it can enter the brain. On the basis of our calculations and assumptions, it appears that the contrast media will have a significant permeability across the blood-brain barrier, and that differences in their permeability may be enough to account for the correlation between partition coefficient and neurotoxicity.

RELATION OF LD_{50} OF SUBSTITUTED BENZOIC ACIDS TO OCTANOL/WATER PARTITION COEFFICIENT AND pK_a

LD_{50} is an administered quantity of drug that is lethal to 50 per cent of test animals. Its measurement alone leaves open the mode of death and the target organs affected, and therefore cannot be used without further information to indicate neurotoxicity. For a large number of drugs, LD_{50} decreases with increasing lipid solubility, indicating that the passage of agents across lipid cell membranes is an important factor in systemic toxicity.[9]

Figure 2 shows that systemic toxicity in mice, as measured by the quantity log $(1/LD_{50})$, is highly correlated with calculated log P_{HA} or log P_A for a large number of benzoic acid derivatives which are analogs of drugs used clinically as contrast media.[38] The major exceptions to this rule are 2-amino- and 4-amino-3,5-diiodobenzoic acid and 3-amino-2,4,6-triiodobenzoic acid, probably because their pK_a's are higher than those of the other acids. The pK_a's of

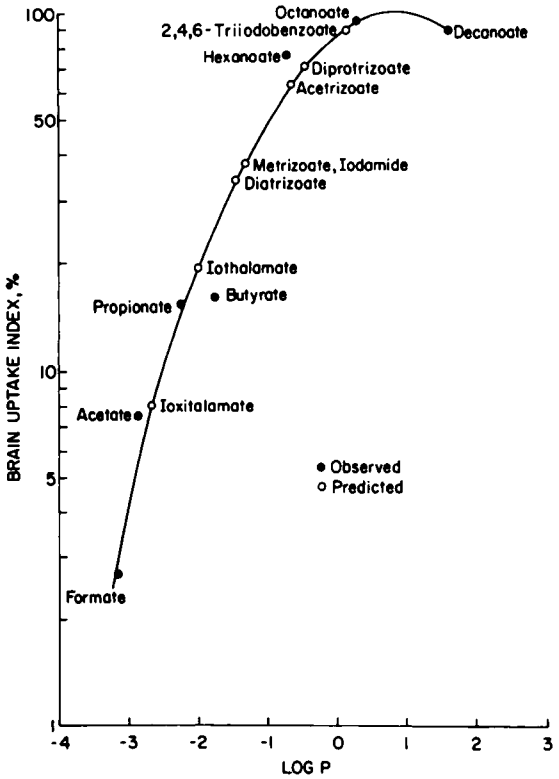


FIG. 2. *Relation of systemic toxicity to log* P_{HA} *for derivatives of benzoic acid.* Systemic toxicity is represented as log ($1/LD_{50}$) for mice.[38] Log P_{HA} is calculated as shown in text. The curve is the least squares fit to the data, excluding points (1), (2) and (3). It is given by the equation, log ($1/LD_{50}$) $= -0.076$ (log P_{HA})$^2 + 1.17$ log $P_{HA} - 4.278$. The numbers represent the following benzoic acid derivatives: (1) 2-amino-3,5-diiodobenzoic; (2) 3-amino-2,4,6-triiodobenzoic; (3) 4-amino-3,5-diiodobenzoic; (4) 2-acetylamino-3,5-diiodobenzoic; (5) 2-butyrylamino-3,5-diiodobenzoic; (6) 2-(n-caproylamino)-3,5-diiodobenzoic; (7) 2-benzoylamino-3,5-diiodobenzoic; (8) 2-(α-phenylbutyrylamino)-3,5-diiodobenzoic; (9) 2-(o-iodobenzoylamino)-3,5-diiodobenzoic; (10) 3-formylamino-2,4,6-triiodobenzoic; (11) 3-acetylamino-2,4,6-triiodobenzoic; (12) 3-propionylamino-2,4,6-triiodobenzoic; (13) 3-butyrylamino-2,4,6-triiodobenzoic; (14) 3-benzoylamino-2,4,6-triiodobenzoic; (15) 3-isobutyrylamino-2,4,6-triiodobenzoic; (16) 3-caproylamino-2,4,6-triiodobenzoic; (17) 3-caprylamino-2,4,6-triiodobenzoic; (18) 3-lauroylamino-2,4,6-triiodobenzoic; (19) 4-acetylamino-3,5-diiodobenzoic; (20) 4-benzylamino-3,5-diiodobenzoic.

Substances (1), (2) and (3) were excluded from the least squares calculation because their pK_a's differ markedly from those of the others (see text). If pK_a's are taken into account by plotting log ($1/LD_{50}$) against log P rather than log P_{HA}, the positions of (1), (2) and (3) would move to the right, relative to the positions of the other compounds, as illustrated by the open circles and arrows in the figure.

the latter are close to 3.0, the value of acetrizoic acid, while the pK_a of 2-amino-benzoic acid is 6.97, of 3-amino-benzoic acid is 4.78 and of 4-amino-benzoic acid is 4.92[15,39] (see Discussion).

A plot of $1/LD_{50}$ against log P, which is calculated with use of these pK_a's (Equation 7), reduces the discrepancies in Figure 2 for compounds (1), (2) and (3). We illustrate this in Figure 2 by showing how the positions of these 3 acids would approximate those of the others, if all $1/LD_{50}$ were plotted against log P rather than log P_{HA}. Differences of pK_a lead to differences in the additive constant of Equation 7.

DISCUSSION

Many factors have been related to the neurotoxicity of x-ray contrast media. Implicated have been agglutination of red blood cells and increased blood viscosity, vasospasm and modification of cerebral blood flow, the direct toxic effect on cells and opening of the blood-brain barrier.[1]

The data reported in this paper show that neurotoxicity of several contrast media with approximately equal pK_a's is correlated with their lipid solubility as measured by their octanol/water partition coefficient P_{HA}. If the pK_a's of the organic acids differ however, brain entry will depend also on the relative concentrations of A^- and HA. These are determined by pK_a and are accounted for in the partition parameter P (Appendix). Organic acids with higher pK_a's have higher proportions of the undissociated HA form, higher values of P and higher systemic toxicities, as illustrated in Figure 2.

Although it is generally believed that the blood-brain barrier excludes charged compounds and the dissociated form of organic electrolytes from entering the brain,[3,28]

these compounds will enter the brain to some degree if they have a finite lipid solubility. The partition coefficient of A^- amounts to $10^{-4.1}$ that of HA for benzoic acid derivatives. Since $pK_a \simeq 3.0$, $[A^-]/[HA] = 10^{4.4}$ at pH 7.4 (Equation 4). The term which determines the relative contributions of A^- and HA to P in Equation 3 is $P_A[A^-]/P_{HA}[HA]$ and equals 2.0. In other words, the dissociated acid form A^- of the contrast media should make up two-thirds of the partition parameter P. It remains to determine actual values of P for the contrast media, as well as their brain uptake index.

For the aliphatic monocarboxylic acids on the other hand, with $pK_a \simeq 4.8$, the quantity $P_A[A^-]/P_{HA}[HA] = 0.03$. Thus, the dissociated form A^- contributes only 3 per cent to P.

The permselectivity of the blood-brain barrier can be reduced by osmotically shrinking cerebrovascular endothelial cells and opening tight junctions between them. Concentrated solutions of urea and NaCl, as well as of contrast media, probably open the blood-brain barrier in this way.[29,30,31,34] It is reasonable to assume that the neurotoxicity which increases as the quantity of contrast medium administered is increased depends in part on osmotic opening of the barrier and the consequent entry of the contrast medium into the brain.

We therefore can identify 2 factors related to neurotoxicity which have to do with passage of contrast medium from blood to brain across the blood-brain barrier. The anion specific factor is the lipid solubility, or partition coefficient of the contrast medium, which determines passage *through* cerebrovascular endothelial cell membranes of the unaltered barrier. The nonspecific factor is the osmolality of the contrast media, which can open tight junctions and permit passage *between* cells.

Both of these influences would be enhanced by prolonged contact with the cerebrovascular system due to prolonged duration of perfusion or to repeated injections, and could explain the enhanced neurotoxicity under these conditions. Additional factors such as reduced blood flow may be important. Possible enhanced entry into the brain by carrier-mediated transport also must be considered.[25]

On the basis of our calculations, it would appear that the neurotoxicity of contrast media could be reduced by reducing P_{HA}. In addition, a reduction in pK_a would decrease the proportion of the more lipid soluble HA form in solution. Neurotoxicity could be reduced also by increasing protein binding,[18,21] although fractional binding is correlated with P_{HA}.[11] The use of polymeric contrast media will reduce medium osmolality[12] and the possible osmotic opening of the blood-brain barrier that also can produce neurotoxicity.

How toxicity is produced once the agents enter the brain is still an unresolved question. The basis for their action may lie in their ability to directly affect central nervous system neurons. Benzoic acid derivatives have been shown to affect the membrane permeability and electrical activity of isolated neurons with a relative potency which is highly correlated with their lipid solubility.[23] Hoppe,[14] however, found no difference in LD_{50} between acetrizoate and diatrizoate when given intracerebrally in mice, although acetrizoate was twice as toxic as metrizoate by the intravenous route. Clearly, the differential effects of contrast media on isolated cells require further study.

SUMMARY

Neurotoxicity of x-ray contrast media used in cerebral and spinal cord angiography is related to 2 factors.

The specific factor is the lipid solubility, or octanol/water partition coefficient of the contrast medium anion, which determines its passage through membranes of cerebrovascular endothelial cells of the blood-brain barrier.

The nonspecific factor is solution osmolality, which opens tight junctions between the cells through which the contrast media then can enter the brain.

Differences in neurotoxicity of 8 contrast media that are derivatives of benzoic acid are correlated with differences in their specific octanol/water partition coefficient. Brain uptake depends on the partition coefficient and on pK_a, which determines the proportion of dissociated and undissociated form of the acid in blood. Systemic LD_{50} of the benzoic acid derivates also depends on octanol/water partition and on pK_a.

S. I. Rapoport, M.D.
Laboratory of Neurophysiology
National Institute of Mental Health
Bethesda, Maryland 20014

APPENDIX

The octanol/water partition coefficient of the undissociated form of an organic acid is defined as P_{HA}, and that of the dissociated form is defined as P_A. Let a combined partition coefficient P depend linearly on the fractional concentrations of A^- and HA in solution, multiplied by their respective partition coefficients,

$$P = P_{HA}\frac{[HA]}{[A^-]+[HA]} + P_A\frac{[A^-]}{[A^-]+[HA]}. \quad (3)$$

This equation means that the contributions to P of A^- and HA are in the ratio $P_A[A^-]/P_{HA}[HA]$. [HA] can be calculated from the acidic dissociation constant K_a and $[H^+]$, and can be written in terms of pK_a and pH as well,

$$\frac{[HA]}{[A^-]} = \frac{[H^+]}{K_a} = 10^{(pK_a-pH)}. \quad (4)$$

When $pH - pK_a > 1$, the acid is at least 90 per cent dissociated and

$$[A^-] \simeq [HA] + [A^-]. \quad (5)$$

Substituting Equations 4 and 5 into Equation 3 gives the following:

$$P = P_{HA}(P_A/P_{HA} + [H^+]/K_a). \quad (6)$$

As discussed in the text, for the benzoic acids and aliphatic monocarboxylic acids $P_A = 10^{-4.1} P_{HA}$, and substitution into Equation 6 gives, after conversion to logarithms,

$$\log P = \log P_{HA} + \log [10^{-4.1} + 10^{(pK_a-pH)}]. \quad (7)$$

At pH 7.4 and when $pK_a = 4.8$ (aliphatic monocarboxylic acids), Equation 7 gives,

$$\log P = \log P_{HA} - 2.59. \quad (8)$$

At $pH = 7.4$ and $pK_a = 3.0$ (benzoic acid derivatives), Equation 7 gives,

$$\log P = \log P_{HA} - 3.92. \quad (9)$$

Equations 8 and 9 differ by a constant.

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REFERENCES

1. ALMÉN, T. Toxicity of radiocontrast agents. In: Radiocontrast Agents. Volume 2. Edited by P. K. Knoefel. Pergamon Press, New York, 1971, p. 443.
2. BLOOR, B. M., WRENN, F. R., and MARGOLIS, G. Experimental evaluation of certain contrast media used for cerebral angiography. *J. Neurosurg.*, 1951, *8*, 585–594.
3. BRODIE, B. B., and HOGBEN, C. A. M. Some physico-chemical factors in drug action. *J. Pharm. & Pharmacol.*, 1957, *9*, 345–380.
4. COLLANDER, R. Permeability of plant protoplasts to non-electrolytes. *Trans. Faraday Soc.*, 1937, *33*, 985–990.
5. CRONE, C. Permeability of brain capillaries to non-electrolytes. *Acta physiol. scandinav.*, 1965, *64*, 407–414.
6. FUJITA, T., IWASA, J., and HANSCH, C. New substituent constant, II, derived from partition coefficients. *J. Am. Chem. Soc.*, 1964, *86*, 5175–5180.
7. HANSCH, C. Personal communication.
8. HANSCH, C. Quantitative structure-activity relationships in drug design. In: Drug Design. Volume 1. Edited by E J Ariens. Academic Press, New York, 1971, p. 271.
9. HANSCH, C., and DUNN, W. J. Linear relationships between lipophilic character and biological activity. *J. Pharm. Sc.*, 1972, *61*, 1–19.
10. HANSCH, C., and GLAVE, W. R. Structure-activity relationships in membrane-perturbing agents: hemolytic, narcotic and antibacterial compounds. *Mol. Pharmacol.*, 1971, *7*, 337–354.
11. HELMER, F., KIEHS, K., and HANSCH, C. Linear free-energy relationship between partition coefficients and binding and conformational

perturbation of macromolecules by small organic compounds. *Biochemistry*, 1968, *7*, 2858–2863.

12. Hilal, S. K. Trends in preparation of new angiographic contrast media with special emphasis on polymeric derivatives. *Invest. Radiol.*, 1970, *5*, 458–472.
13. Hoey, G. B., Wiegert, P. E., and Rands, R. D. Organic iodine compounds as x-ray contrast media. In: Radiocontrast Agents. Volume 1. Edited by P. K. Knoefel. Pergamon Press, New York, 1971, p. 23.
14. Hoppe, J. O., Some pharmacological aspects of radiopaque compounds. *Ann. New York Acad. Sc.*, 1959, *78*, 727–739.
15. Hoppe, J. O. Duprey, L. P., Borisenok, W. A., and Bird, J. G. Selective radiopacity in cardiovascular angiography. *Angiology*, 1967, *18*, 257–272.
16. Jaffé, H. H. Reexamination of Hammett equation. *Chem. Rev.*, 1953, *53*, 191–261.
17. Jeppsson, P. G., and Olin, T. Neurotoxicity of roentgen contrast media: study of blood-brain barrier in rabbit following selective injection of contrast media into internal carotid artery. *Acta radiol.* (Diag.), 1970, *10*, 17–34.
18. Knoefel, P. K. Binding of iodinated radiocontrast agents to plasma proteins. In: Radiocontrast Agents. Volume 1. Edited by P. K. Knoefel. Pergamon Press, New York, 1971, p. 133.
19. Krogh, A. Active and passive exchanges in inorganic ions through surfaces of living cells and through living membranes generally. *Proc. Roy. Soc. Ser. Biol.*, 1946, *133*, 140–200.
20. Lampe, K. F., James, G., Ebersfeld, M., Mende, T. J., and Viamonte, M. Cerebrovascular permeability of water-soluble contrast material, hypaque (sodium diatrizoate): experimental study in dogs. *Invest. Radiol.*, 1970, *5*, 79–85.
21. Lang J. H., and Lasser, E. C. Binding of roentgenographic contrast media to serum albumin. *Invest. Radiol.*, 1967, *2*, 396–400.
22. Leo, A., Hansch, C., and Elkins, D. Partition coefficients and their uses. *Chem. Rev.*, 1971, *71*, 525–616.
23. Levitan, H., and Barker, J. L. Salicylate: structure-activity study of its effects on membrane permeability. *Science*, 1972, *176*, 1423–1425.
24. Nickel, A. R. Personal communication.
25. Oldendorf, W. H. Carrier-mediated blood-brain barrier transport of short-chain monocarboxylic organic acids. *Am. J. Physiol.*, 1973, *224*, 1450–1453.
26. Olin, T. B., and Redman, H. C. Experimental evaluation of contrast media in vertebral circulation. *Acta radiol.*, 1967, Suppl. 270, pp. 216–227.
27. Overton, E. Über die Osmotischen Eigenschaften der Lebenden Pflanzen und Tierzelle. Vierteljahresschr. Naturforsch. Ges. Zürich, 1895, *40*, 159–201.
28. Rall, D. P., Stabenau, J. R., and Zubrod, C. G. Distribution of drugs between blood and cerebrospinal fluid: general methodology and effect of pH gradients. *J. Pharmacol. & Exper. Therapy*, 1959, *125*, 185–193.
29. Rapoport, S. I. Evidence for reversible opening of blood-brain barrier by osmotic shrinkage of cerebrovascular endothelium and opening of tight junctions: relation to carotid arteriography. In: Small Vessel Angiography: Imaging, Morphology, Physiology and Clinical Applications. S. K. Hilal, Editor. C. V. Mosby Company, St. Louis, 1973, p. 137.
30. Rapoport, S. I., Hori, M., and Klatzo, I. Testing of hypothesis for osmotic opening of blood-brain barrier. *Am. J. Physiol.*, 1972, *223*, 323–331.
31. Rapoport, S. I., Brightman, M. W., and Reese, T. S. Reversible osmotic opening of blood-brain barrier (BBB) by opening of tight junctions of cerebrovascular endothelium. *Abstracts, 17th Annual Meeting Biophysical Soc.*, 1973, *13*, 230a.
32. Rapoport, S. I., and Levitan, H. Dependence of neurotoxicity of x-ray contrast media on lipid solubility. *Abstracts, 11th Annual Meeting Am. Soc. Neuroradiology*, 1973, p. 4.
33. Rapoport, S. I., and Thompson, H. K. Osmotic opening of blood-brain barrier in monkey without associated neurological deficits. *Science*, 1973, *180*, 971.
34. Rapoport, S. I., Thompson, H. K., and Bidinger, J. M. Equi-osmolal opening of blood-brain barrier in rabbit by different x-ray contrast agents. *Acta radiol.* (Diag.), 1974, *15*, 21–32.
35. Reese, T. S., and Karnovsky, M. J. Fine structural localization of blood-brain barrier to exogenous peroxidase. *J. Cell. Biol.*, 1967, *34*, 207–217.
36. Tindall, G. T., Kenan, P. D., Phillips, R. L., Margolis, G., and Grimson, K. S. Evaluation of roentgen contrast agents used in cerebral arteriography: II. Application of new method. *J. Neurosurg.*, 1958, *15*, 37–44.
37. Tornell, G. Bradycardial reactions in cerebral angiography induced by sodium and methylglucamine iothalamate (Conray). *Acta radiol.* (Diag.), 1968, *7*, 489–501.
38. Wallingford, V. H., Decker, H. G., and Kruty, M. X-ray contrast media: I. Iodinated acylaminobenzoic acids. *J. Am. Chem. Soc.*, 1952, *74*, 4365–4368.
39. Weast, R. C. Editor. Handbook of Chemistry and Physics. Fifty-first edition. Chemical Rubber Company Press, Cleveland, 1970.