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Protein Binding of Salicylic and Salicyluric Acid in Serum from Malnourished Children: The Influence of Albumin, Competitive Binding and Non-esterified Fatty Acids

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Abstract—The serum protein binding of salicylic and salicyluric acid has been determined by ultrafiltration in 60 children after administration of oral salicylate. The children were classified according to nutritional status: well-nourished (n = 12), underweight (n = 12), marasmic (n = 17) marasmic-kwashiorkor (n = 7) and kwashiorkor (n = 12). Salicylic acid free fractions were 0·106 ± 0·026, 0·114 ± 0·069, 0·141 ± 0·037, 0·285 ± 0·279 and 0·438 ± 0·190 in the five groups, respectively. Salicyluric acid free fractions were 0·184 ± 0·057, 0·280 ± 0·282, 0·236 ± 0·114, 0·484 ± 0·497 and 0·646 ± 0·261, respectively. The degree of binding was dependent on serum albumin levels, ligand concentrations and non-esterified fatty acids (NEFA). The NEFA/albumin ratio ranged from 0·05 to 6·6. The fitting of a one-site Scatchard binding model to the collective data was improved when a decrease was allowed for in the number of binding sites in proportion to NEFA concentrations. Salicyluric acid binding could be fitted only when inhibition of the parent compound was included. Binding was not affected by age or sex. The major determinants of salicylate binding in sera from malnourished children have thus been identified.

Childhood protein-energy malnutrition is a complex syndrome defying explicit classification (Waterlow & Alleyne 1971; Coward & Lunn 1981). Adaptation to deficient protein-energy food intake causes biochemical and physiological changes which may influence the disposition of drugs and other exogenous compounds (Krishnaswamy 1978). The common observation of hypoalbuminaemia in malnourished subjects may cause a decreased binding of drugs with affinity to this protein. Diminished binding has been observed in plasma taken from malnourished subjects after administration of doxycycline (Raghuram & Krishnaswamy 1982), phenylbutazone (Krishnaswamy et al 1981) and sulphafurazole (Shastri 1980). In-vitro equilibrium dialysis on pooled serum from hospitalized children suffering from kwashiorkor has shown no or little difference in binding compared with normal serum binding to 18 drugs, most of which exhibited low to moderate degrees of binding. However, with flucloxacillin and cloxacillin, 75% increase in unbound fractions was observed (Buchanan 1977).

Salicylic acid (mol. wt 138.12, $pK_a = 3·0$) has a wide use as an antipyretic, analgesic and anti-inflammatory agent. Its elimination is mainly metabolic, some of the conjugative pathways being saturable. Salicylate binding to serum albumin is non-linear at therapeutic concentrations (Furst et al 1979). A previous study on salicylic acid binding in malnourished children showed that saturation of binding occurred at lower concentrations in spiked pooled kwashiorkor serum compared with normal serum (Eyberg et al 1974). Salicylate binding was decreased in spiked serum from rats fed a low protein-calorie diet over three weeks (Yue & Varma 1982).

As part of a study on the disposition of salicylic acid in malnourished children, we have sought to elucidate the major determinants of salicylate serum protein binding in this group of patients. We have investigated the influences of serum albumin, non-esterified fatty acids (NEFA) and ligand concentrations.

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Materials and Methods

Subjects

Sixty inpatients at the Ethio-Swedish Childrens' Hospital were given sodium salicylate in the course of their treatment. The drug was given in the morning with food intake restricted until 1·5–2 h had elapsed after administration. Nutritional status was classified according to the weight for age and the presence of oedema in combination with low serum protein (Editorial, Lancet 1970). Patients in an unstable circulatory condition or a dehydrated state were excluded from the study. Patient data is summarized in Table 1. Only one patient was on record as having received another drug with serum binding above 90% within the previous 24 h (cloxacillin).

Ethical procedures

Sampling was undertaken after parental or guardian consent had been given. The study was approved by the Ethics Committee of the Karolinska Institute.

Sampling

One and a half to 5 h after oral administration of either 12·5 or 25 mg kg^{-1} sodium salicylate in water, one sample of 1–2

Table 1. Patient data and unbound fractions for salicylic and salicyluric acid (mean ± s.d.) after a single salicylic acid dose as per nutritional group.

	n	Age (years)	Sex (F/M)	Weight (kg)	Hct (%)	T. S-protein ($g\ L^{-1}$)	S-albumin ($g\ L^{-1}$)	NEFA (μequiv L^{-1})	Unbound concns (μM)		Fraction unbound	
									Salicylic acid	Salicyluric acid	Salicylic acid	Salicyluric acid
Well-nourished	12	3·7 ± 1·3	4F/8M	13·4 ± 2·3	38·6 ± 5·0	72·9 ± 4·7	41·0 ± 5·6	237 ± 193	47·1 ± 22·9	2·77 ± 3·56a	0·106 ± 0·026	0·184 ± 0·057(a)
Underweight	12	3·4 ± 1·7	4F/8M	10·0 ± 3·6**	40·1 ± 3·3	73·2 ± 5·5	37·6 ± 6·8	324 ± 328	37·1 ± 32·3	2·09 ± 1·58	0·114 ± 0·069	0·280 ± 0·282
Marasmic	17	3·3 ± 2·0	9F/8M	7·9 ± 3·2***	36·7 ± 7·6	69·9 ± 8·9	34·2 ± 5·9**	492 ± 589	45·7 ± 32·0	1·96 ± 0·82b	0·141 ± 0·037	0·236 ± 0·114(b)
Marasmic-kwashiorkor	7	2·6 ± 1·6*	6F/1M	7·7 ± 1·4***	31·4 ± 3·7**	62·3 ± 14·5*	26·6 ± 9·1***	420 ± 295	109 ± 91·9*	3·62 ± 0·93**	0·285 ± 0·279*	0·484 ± 0·497*
Kwashiorkor	12	2·5 ± 1·3*	6F/6M	9·3 ± 3·2***	35·5 ± 3·9*	46·8 ± 11·5***	19·8 ± 7·8***	779 ± 446***	110 ± 32·5***	5·34 ± 2·50***	0·438 ± 0·190***	0·639 ± 0·250***

(a) n = 10, (b) n = 16. Medians were tested against the Well-nourished group (Mann-Whitney U-test).
* = $0{\cdot}01 < P \leqslant 0{\cdot}05$; ** = $0{\cdot}001 < P \leqslant 0{\cdot}005$; *** = $P \leqslant 0{\cdot}001$.

mL venous blood was drawn from each patient by polyethylene syringe and transferred to a non-heparinized glass test-tube where it was allowed to clot over 30–60 min at room temperature (20°C). The varying times of collection were to provide an extended range of drug and metabolite concentrations. After centrifugation, serum was transferred to polyethylene microtubes and kept frozen until assayed.

Chemical assay

Salicylic (SA) and salicyluric acid (SAU) were determined by HPLC. A 50 μL serum aliquot was transferred to a microtube, to which 10 μL 35% perchloric acid and 50 μL methanol were added. After agitation and centrifugation, a sample of the clear supernatant (50 μL) was injected onto a C_{18} analytical column. The mobile phase (12% acetonitrile, 15% methanol in phosphate buffer, pH 7·0, containing 0·5 mM tetrabutylammonium sulphate) allowed the separation of drug and metabolite. The eluate was monitored at 305 nm, the UV detector output being led to an integrator providing peak areas. Standard plasma samples were acquired by spiking blood bank human plasma with sodium salicylate (Merck, Darmstadt, F.R. Germany) and salicyluric acid (Sigma, St Louis, USA). Assay reproducibility for salicylic and salicyluric acid is shown in Table 2.

Table 2. Assay reproducibility for salicylic and salicyluric acid in water solution and spiked human serum. Peak areas determined by runs of 10 samples at each concentration. Coefficient of variation (CV) = 100*Standard Deviation/Mean. The lower limit of determination may be seen as the concentration at which the CV exceeds 15%.

	Concn (μM)	Water CV	Serum	
			CV	Recovery (%)
Salicylic acid	3·25	14·5	36·5	106
	541·7	2·3	2·0	92
	1119·4	1·7	2·0	95
Salicyluric acid	1·94	6·1	15·1	135
	161·8	3·7	3·8	101
	323·5	2·4	2·2	97

A simple nephelometric method was used to estimate total serum proteins. Serum albumin was determined spectrophotometrically in duplicate with a bromocresol green method as modified by Doumas et al (1971). The molecular weight of the standard bovine albumin (Fraction V, United States Biochemical Corporation, Cleveland, Ohio) was taken as 69 000 daltons. Total serum NEFA was assayed with an enzymatic colorimetric test kit (NEFAC;—WAKO Chemicals GmbH, Neuss, FRG), scaled down to a sample volume of 25 μL. All samples were corrected for specimen blanks to avoid interference of haemoglobin, lipids and bilirubin. Total NEFA concentrations were expressed as equivalents of oleic acid, it being the external standard supplied. Salicylate (500 μM NaSA in water solution) did not absorb at the wavelength used (550 nm).

Protein binding

Protein binding was determined by ultrafiltration (Amicon Micropartition System MPS-1 with YMT membranes). Samples placed for 15 min in a waterbath at 37°C were lightly gassed with carbogen (5% CO_2:95%O_2), evaporation being

avoided. After measurement of pH with indicator paper, a 300 μL aliquot was transferred to the ultrafiltration tube and more gas used before sealing with the cap. The tubes were spun in the outer circle in a fixed angle SM-24 rotor and Sorvall RC-5B chamber for 6 min at 900 *g* (3000 rev min^{-1}). All equipment was preheated and chamber temperature was kept as close as possible to 37°C during runs. The filtrate was assayed as above but without the addition of perchloric acid or methanol against a water solution calibration curve; 25 μL was injected into the HPLC column.

Spiked human serum samples (4% v/v dilution) were filtered to yield 15, 30 and 50% of sample volume, each at four different pH values (7·0, 7·4, 7·8 and 8·1; glass electrode) and at the two concentrations of 43·3:25·9 μM SA:SUA and 952·3:129·4 μM SA:SUA, respectively. Since no systematic trends were evident, patient samples were subsequently filtered to yield 27·3 ± 5·1% of sample volumes. The mean pH in patient samples was 8·0 ± 0·4 when measured by indicator paper, which we found to overestimate pH values, probably due to loss of carbon dioxide during measurement. There was no evidence of loss of drug to membrane in the range of concentrations or filtration volumes studied. The thawing of spiked samples up to four times did not affect the free fractions determined for SA or SUA.

Data analysis

Scatchard type and stepwise equilibrium models were fitted to the data using MAXFIT (Biomedical Systems Analysis, Uppsala University), a maximum likelihood parameter estimation program run on a BASF 8/85 (IBM 381 compatible) mainframe computer. Data were weighted by $1/(P_1 \cdot C_{calc}^{P2})^2$. The variance parameters P1 and P2 were estimated by the program. When two functions were fitted simultaneously, each was weighted by its own set of estimated variance parameters. Model discrimination was based primarily on residual plot analysis, coefficients of variation of structural model parameter estimates and log-likelihood values (LogL). Full and reduced modes were compared by calculation of $F = LogL_{reduced} - LogL_{full}$ according to Sheiner (1986). Only salicylic acid data were initially used to select a primary structural model. Fits were challenged by alternative sets of initial values to prevent convergence at local minima.

A 1-site Scatchard type model proved adequate for this data set and was not improved upon by stepwise equilibrium models nor multiple-site Scatchard type models. The following equation was therefore used (Model I):

$$C = C_u + \frac{N^*Pt^*K^*C_u}{1 + K^*C_u} \quad (1)$$

where the total concentration (C) is dependent on the unbound ligand concentration (C_u), the (average) number of binding sites per mole protein (N), the total concentration of binding protein (Pt), here taken as serum albumin, and the apparent association constant between ligand and binding site (K).

The binding of salicylic and salicyluric acid was fitted both independently and by allowing for competitive binding interactions by simultaneously fitting equations 2 and 3 (Model II):

$$C_{SA} = C_{u,SA} + \frac{N_{SA}^*Pt^*C_{u,SA}^*K_{SA}}{1 + K_{SA}^*C_{u,SA} + K_{SUA}^*C_{u,SUA}} \quad (2)$$

$$C_{SUA} = C_{u,SUA} + \frac{N_{SUA}^*Pt^*C_{u,SUA}^*K_{SUA}}{1 + K_{SUA}^*C_{u,SUA} + K_{SA}^*C_{u,SA}} \quad (3)$$

Residuals plotted against NEFA concentrations gave reason to incorporate free fatty acid inhibition of ligand binding into the model. The best fit was achieved with a model where the total number of binding sites diminished proportionately to NEFA concentration. Upon modification of equation 1 the binding was thus described by (Model III):

$$C = C_u + \frac{(N^*Pt - \alpha^*C_{NEFA})^*K^*C_u}{1 + K^*C_u} \quad (4)$$

where α is a proportionality constant reflecting to what extent NEFA concentrations decrease the total number of available binding sites (N*Pt), and C_{NEFA} is the total concentration of non-esterified fatty acids. By similar insertion into equations 2 and 3, a model describing both NEFA inhibition and competitive binding between drug and metabolite was defined (Model IV):

$$C_{SA} = C_{u,SA} + \frac{(N_{SA}^*Pt - \alpha^*C_{NEFA})^*C_{u,SA}^*K_{SA}}{1 + K_{SUA}^*C_{u,SA} + K_{SUA}^*C_{u,SUA}} \quad (5)$$

$$C_{SUA} = C_{u,SA} + \frac{(N_{SUA}^*Pt - \alpha^*C_{NEFA})^*C_{u,SUA}^*K_{SUA}}{1 + K_{SUA}^*C_{u,SUA} + K_{SA}^*C_{u,SA}} \quad (6)$$

Results

Serum albumin was decreased in malnourishment with kwashiorkor levels being halved compared with the well-nourished group (Table 1). Total serum fatty acids tended to increase with degree of malnutrition, but exhibited a large variation within groups. Median NEFA/albumin ratios were 0·38, 0·33, 0·49, 1·07 and 3·64 in the five groups, respectively, with a range of 0·05–6·6. There was no correlation between NEFA and albumin concentrations.

Salicylic acid-free fractions did not differ between well-nourished, underweight or marasmic patients but were elevated in marasmic-kwashiorkor and kwashiorkor children. Salicyluric acid unbound fractions exhibited a larger variation but followed the same trend as its parent compound. Unbound salicyluric acid concentrations were not detectable in two patients in the well-nourished group and in one patient in the marasmic group. In two marasmic-kwashiorkor patients and one kwashiorkor child, metabolite concentrations were close to the detection limit resulting in free fractions exceeding unity (1·02, 10·8 and 1·37). These were given values of 1·0 when calculating the means. Drug and metabolite free fractions ranged between 0·064–0·79 and 0·10–1·0, respectively.

There was a poor correlation between total and unbound concentrations for both salicylic and salicyluric acid, accentuated by the kwashiorkor group (Fig. 1). There was a marked increase in free fractions with decreasing concentrations of serum albumin (Fig. 2) as well as a tendency towards higher free fractions with increasing unbound ligand concentrations. There was a fair correlation between salicylic and salicyluric acid free fractions ($fu_{SUA} = 1{\cdot}43$ ($\pm 0{\cdot}11$)*$fu_{SA} = 0{\cdot}06$ ($\pm 0{\cdot}03$), $r = 0{\cdot}874$; $n = 57$).

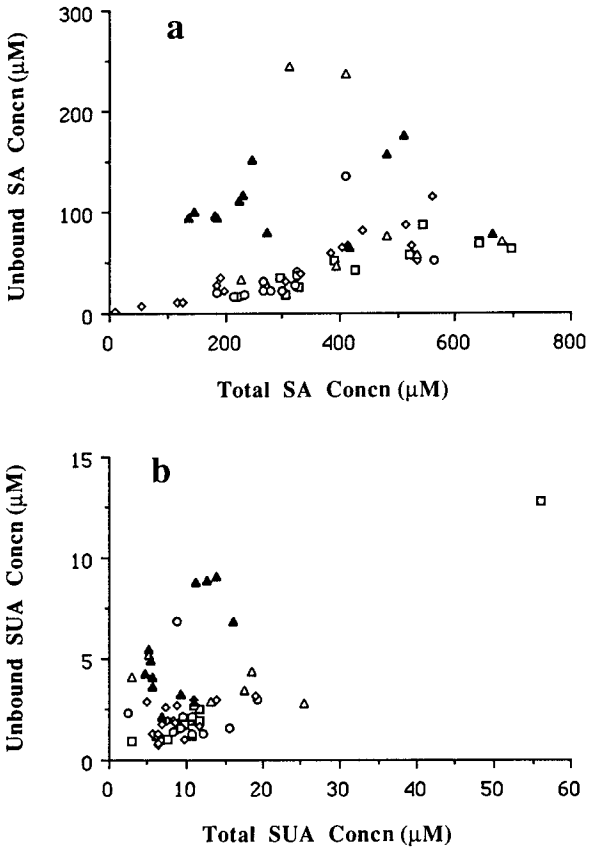


FIG. 1. Relationship between unbound and total concentrations of (a) salicylic and (b) salicyluric acid in sera from children classified as well-nourished (□), underweight (○), marasmic (◇), marasmic-kwashiorkor (△) or kwashiorkor (▲).

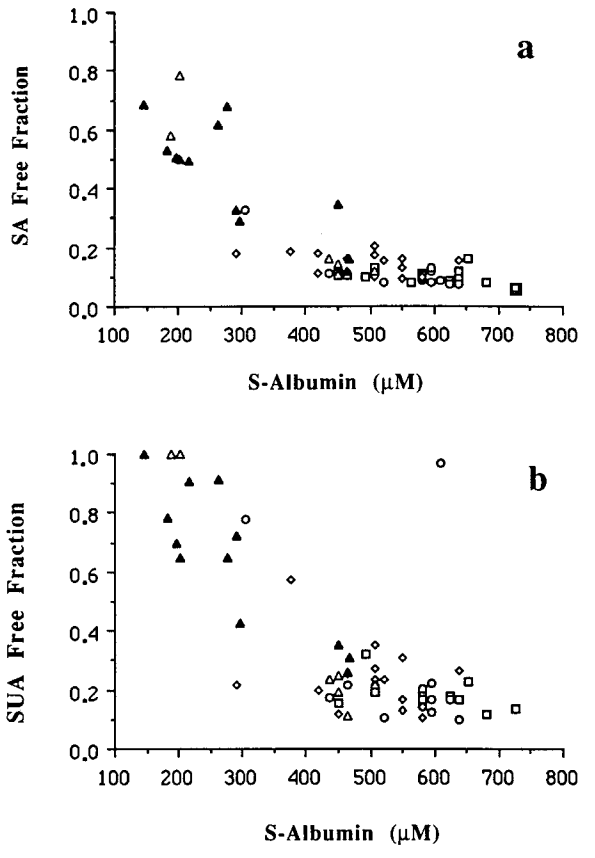


FIG. 2. Free fractions of (a) salicylic and (b) salicyluric acid increase with decreasing serum albumin concentrations. Symbols: well-nourished (□), underweight (○), marasmic (◇), marasmic-kwashiorkor (△) or kwashiorkor (▲). Salicyluric acid free fractions were taken as 1·0 in three samples where estimates exceeded unity.

Resulting parameter estimates for the different models are presented in Table 3. Application of a basic 1-site Scatchard binding model (Model I) underestimated salicylic acid free fractions in kwashiorkor serum samples. The model was not applicable for salicyluric acid. Allowing for competitive inhibition between drug and metabolite (Model II) did not improve the fit for salicylic acid, and underpredicted fractions unbound for salicyluric acid.

Allowing the molar number of binding sites to be diminished in proportion to free fatty acid concentration (Model III) improved the fit for salicylic acid ($P < 0{\cdot}001$; F-test) but was not computable for the metabolite alone. The variation in salicyluric acid binding was best described when competitive binding of parent compound and NEFA inhibition was included as in Model IV. This gave salicylic acid estimates similar to those obtained when fitting drug data alone (Model III), with weighted sum of squares of 59·5 and 60·1, respectively, for salicylic acid. There were no trends in residuals versus independent variables from fits with either Model III and IV, nor when plotted against age, total protein, pH, haematocrit or percent of sample volume filtered. Residuals were evenly distributed between the sexes.

One kwashiorkor and one marasmic-kwashiorkor child exhibited larger salicylic acid free fractions than was predicted by any model; these residuals could not be traced to concomitant medication or any other factor within the scope of the study. In one underweight child an unaccountably high salicyluric free fraction was observed. Excluding these three outliers did not appreciably change parameter estimates (Table 3,IVb). Predicted free fractions showed good correlation with observed values (Fig. 3).

It was not possible to separately fit combined data from kwashiorkor and marasmic-kwashiorkor groups with any precision. Fitting Models III and IV to the combined data from well-nourished, underweight and marasmic patients improved goodness-of-fit compared with models without fatty acid inhibition ($P < 0{\cdot}01$; F-test).

Discussion

Kwashiorkor children, on average, had a four-fold elevation of salicylic free fractions compared with normal children. In contrast, marasmic children exhibited a negligible increase. Some kwashiorkor children had extremely low levels of serum albumin, the protein binding salicylic acid in plasma. Serum albumin was decreased in marasmus, but not to the same extent. Furthermore, concentration-dependent ligand binding could be expected to have the largest influence in the kwashiorkor group where the highest unbound salicylic acid concentrations were found. NEFA tended to increase with severity of malnutrition, the highest levels being found in kwashiorkor children. In combination, these factors

Table 3. Parameter estimates for salicylic (SA) and salicyluric acid (SUA) protein binding in serum samples from children of varying nutritional status fitted collectively. Parameter precisions are given within parentheses as coefficients of variations (CV = 100*Standard Error of Estimate/Parameter Estimate). n = number of observations, N = number of binding sites, K = apparent association constant (mM^{-1}), α = NEFA inhibition proportionality factor. Models: I = basic 1-site Scatchard (not computable for SUA), II = simultaneous fit of SA and SUA with competitive binding, III = basic model with NEFA inhibition (not computable for SUA), IV = simultaneous fit of SA and SUA with competitive binding and NEFA inhibition (IVa: all data, IVb: excluding 3 outliers). Log-log-likelihood values. Model III performed significantly better than Model I, and Model IVa better than Model II (F-test, $P < 0.001$).

Model	n SA; SUA	N, SA	K, SA	N, SUA	K, SUA	α	LogL
I	60	1·47 (2·5)	15·9 (4·6)	—	—	—	−578
II	60; 57	1·49 (3·5)	18·1 (2·7)	0·185 (15·9)	79·74 (19·4)	—	−768
III	60	2·42 (3·0)	9·14 (4·3)	—	—	0·294 (4·2)	−544
IVa	60; 57	2·09 (5·0)	11·5 (7·8)	1·50 (4·8)	9·39 (6·5)	0·244 (5·3)	−716
IVb	57; 54	2·23 (3·3)	10·5 (4·9)	1·57 (2·8)	8·89 (4·4)	0·251 (3·7)	−665

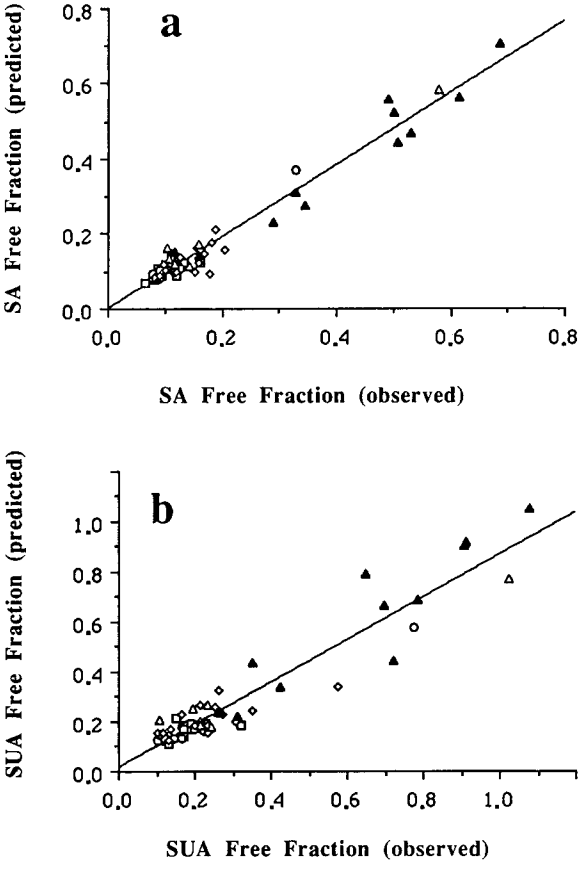


FIG. 3. Predicted versus observed free fractions for (a) salicylic and (b) salicyluric acid resulting from fitting Model IVb. Predicted values were calculated as $C_{u,observed}/C_{calculated}$. Regression slopes were close to unity (0·96 and 0·85 for salicylic and salicyluric acid, respectively) and intercepts close to origin (0·003 and 0·020). Correlation coefficients were 0·98 and 0·95, respectively. Symbols represent samples from well-nourished (□), underweight (○), marasmic (◇), marasmic-kwashiorkor (△) or kwashiorkor (▲) children.

appeared to determine drug binding variability and together contributed to the poor correlation between total and unbound concentrations (Fig. 1). For this reason we sought to model the binding and to determine whether the above variables would suffice to describe the great variation of salicylate binding exhibited in our data.

A number of models have been used to describe the binding of salicylic acid to albumin. These include basic Scatchard type (Borgå et al 1976; Cham et al 1982; Shah et al 1974; Kober et al 1978), and multiple/stepwise equilibrium models (Fletcher & Spector 1977; Aarons et al 1979; Honoré & Brodersen 1984). A one-site Scatchard type model proved to be an adequate, descriptive model for our clinical data. However, the basic model (I) underpredicted free fractions in kwashiorkor children.

We found higher free fatty acid concentrations in kwashiorkor children compared with well-nourished patients. Similar findings previously reported have been explained as a consequence of mobilization of energy resources (Lewis et al 1964). In our study, samples were obtained at variable times after food ingestion, which may influence NEFA levels. Precise determination of these requires immediate assay to avoid lipolysis. As we had no opportunity to assay them until several months after sampling, it would be prudent to suspect that NEFA levels reported herein may be an artifact of sample handling and storage (Ridd et al 1982). However, the mean values for the normal and marasmic groups were slightly below or within the normal range for subjects after overnight fasting, suggesting no gross overestimation of NEFA. Furthermore, serum triglyceride levels are low in kwashiorkor (Lewis et al 1964).

Residual analysis after fitting the basic Scatchard model indicated an influence of NEFA on the binding of salicylic acid. NEFA have been found to both enhance and inhibit drug binding (Naranjo & Sellers 1986). The effect on binding appears to be caused by allosteric effects, as fatty acid molecules, upon binding to albumin, cause a conformational change of the protein (Spector 1975; Maruyama et al 1986). Preliminary efforts on our part to model the influence of NEFA by affecting the association constant, returned imprecise parameter estimates in contrast to when NEFA were allowed to influence the number of binding sites. The magnitude of the proportionality constant α is in agreement with findings that the molar NEFA/albumin ratio has to exceed 2 to 3 in order to diminish the binding of several drugs, including salicylic acid (Schwartz et al 1980). Ratios of this magnitude were found mainly in children suffering from kwashiorkor. Fatty acid inhibition also improved the degree of fits when kwashiorkor and marasmic-kwashiorkor samples were excluded. It seems probable that diminished salicylate binding in severely malnourished children is not a

special case but one exacerbated by high NEFA levels in combination with low serum albumin.

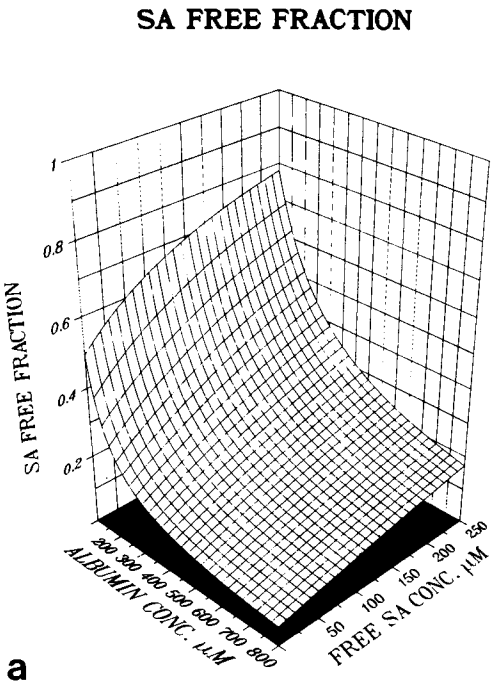
However, other factors may also affect drug protein binding in malnutrition and the interpretation of these results. It is known that fatty acids of different chain lengths may affect binding to different degrees (Maruyama et al 1986). Structural changes in the albumin molecule constitute a possibility although the amino acid composition appears unaltered in kwashiorkor (Potgeiter et al 1967). Bromocresol dye methods for albumin determination may overestimate concentrations, especially in samples containing high levels of acute-phase proteins (Hill 1985). Other investigators have found low serum urea levels in malnutrition (Viteri et al 1964) making translational changes in the albumin molecule (Erill et al 1980) less likely as a source of variation. The decrease in kidney function in severe malnutrition, including diminished acid excretion (Alleyne 1967), may lead to accumulation of endogenous displacers.

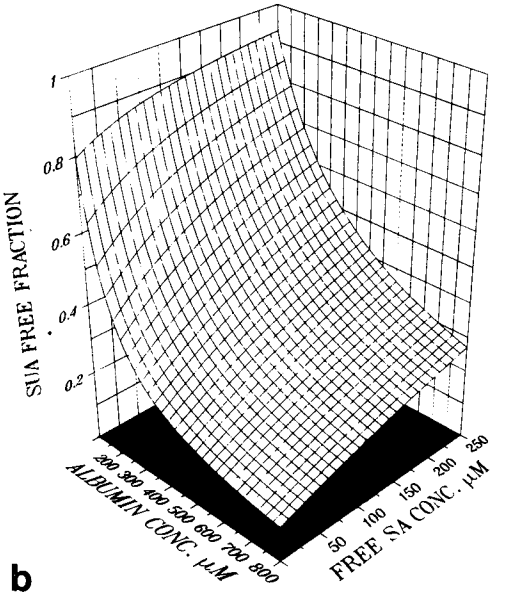
Residuals did not show a trend with age and were evenly scattered between the sexes when using Models III and IV. Windorfer et al (1978) reported that although salicylic acid protein binding was low in prematures, infants aged between one and ten months already had degrees of binding similar to adults, with no differences evident between sexes.

The salicylic acid binding parameter estimates are in agreement with those of other investigators despite different methodologies and data analysis (Borgå et al 1976; Cham et al 1982; Furst et al 1979; Kober et al 1978; Shah et al 1974). However, it is not obvious whether salicylic acid parameters from fitting Models III or IV are those of choice. Since these two models cannot be formally defined in terms of a full and reduced model, normal hypothesis testing is not applicable. The similarity of salicylic acid parameter estimates and weighted sum of squares suggests a choice to be of little value. In general, metabolite inhibition of salicylic binding may be expected to be of negligible importance.

The correlation between salicylic and salicyluric acid free fractions indicated common binding characteristics. Salicyluric acid data could not be fitted unless an allowance was made for competitive binding. It is therefore likely that the parent drug is able to displace its metabolite. With the models tested, salicyluric acid free fractions were slightly underpredicted with a larger scatter between observed and predicted values than for salicylic acid. Binding parameters appeared to be similar to those of the parent compound. However, analytical error at the low concentrations measured suggested caution in evaluation. In the patient with the highest NEFA/albumin ratio of 6·6, a predicted salicyluric acid free fraction above unity resulted. This illustrated that, with the present parameter estimates, the predictive capability of Model IV was lost as NEFA/albumin ratios approached 6 and 9 for salicyluric and salicylic acids, respectively.

In conclusion, the protein binding of salicylic and salicyluric acid after oral administration of sodium salicylic acid was studied in 60 children of varying nutritional status. Drug and metabolite unbound fractions varied 10-fold between subjects. Binding capacities were reduced in severely malnourished patients. A binding model was sought to describe the great variation encountered in our data. A Scatchard model incorporated the influence of serum albumin and





ligand concentrations. Model modification by allowing for non-esterified fatty acid binding inhibition excellently described salicylic acid binding. A correlation was found between salicylic and salicyluric acid binding degrees. The possibility of parent drug displacement of metabolite binding was inferred from a competitive model improving the fits for salicyluric acid. With several variables determining salicylate binding, simple linear correlation in order to predict unbound from total concentrations was excluded (Fig. 1). Fig. 4 illustrates the influence of several variables on salicylic and salicyluric acid protein binding. In a clinical

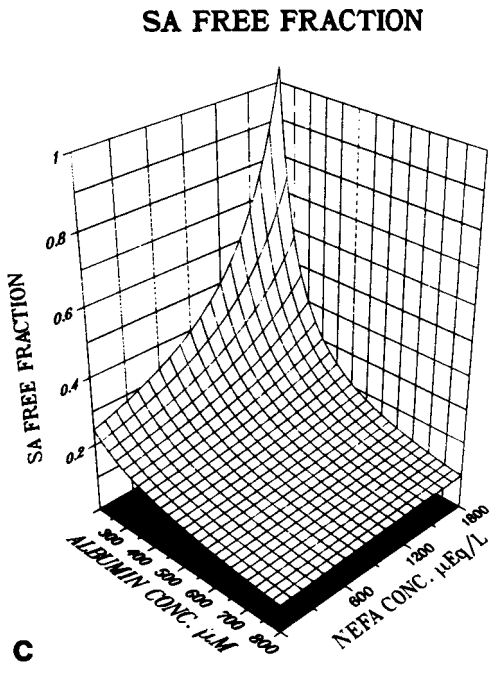


FIG. 4. Graphical representation of salicylate binding. Simulations based on binding parameters from fit IVb. (a): Combined influence of albumin and unbound ligand concentrations on salicylic acid free fraction ($C_{NEFA} = 500$ μequiv L^{-1}, $C_{u,SUA} = 0$). (b): Salicyluric acid binding dependency on albumin and salicylic acid concentrations ($C_{NEFA} = 500$ μequiv L^{-1}, $C_{u,SUA} = 10$ μM). (c): Elevated salicylate free fractions are predicted only at high NEFA concentrations in combination with low serum albumin. Depicted for salicylic acid when $C_{u,SUA} = 0$ and $C_{u,SA} = 50$ μM. The model does not allow for extrapolations to NEFA/albumin ratios approaching 9.

context a word of caution is in place as to the interpretation of these graphs. An elevated free fraction does not itself necessitate adjustment of a dosage regimen. Average unbound concentrations after repeated doses will primarily depend on hepatocellular activity (unbound clearance).

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