The Pharmacology of Malnutrition

PART I. SALICYLATE BINDING STUDIES USING NORMAL SERUM/PLASMA AND KWASHIORKOR SERUM

C. EYBERG, G. P. MOODLEY, N. BUCHANAN

SUMMARY

The binding of salicylate to serum and plasma has been reinvestigated. At normal pH, binding was saturated at about 40 mg/100 ml of total serum salicylate. In kwashiorkor serum, binding was saturated at approximately 15 mg/100 ml. The implications of the latter finding are discussed.

S. Afr. Med. J., **48**, 2564 (1974).

We recently suggested that in infantile salicylism, the amount of salicylate in the cerebrospinal fluid (CSF) is of prime pathological importance. The CSF salicylate concentration, in turn, is closely related to the free serum salicylate concentration.[1,2]

Metabolic and Nutrition Research Unit, Department of Paediatrics, Baragwanath Hospital and University of the Witwatersrand, Johannesburg

C. EYBERG, B.SC.
G. P. MOODLEY, D.M.T.
N. BUCHANAN, M.B. B.S., F.C.P. (S.A.)

Date received: 3 June 1974.
Reprint requests to: Dr N. Buchanan, Metabolic Unit, Baragwanath Hospital, P.O. Bertsham, Johannesburg.

Although salicylate binding has been extensively studied over the past three decades,[3] it was felt worth while to reinvestigate the problem, using a new dialysis system. At the same time, the binding of salicylate to hypoalbuminaemic kwashiorkor serum was studied. The results of these studies are reported here.

MATERIALS AND METHODS

Plan of Study

Fresh pooled adult serum was loaded with sodium salicylate at various concentrations and then dialysed; the same procedure was followed with fresh pooled adult plasma. The effects of pH were studied by the addition of hydrochloric acid or sodium bicarbonate, so as to obtain pH levels of 6,97; 7,5 and 8,15.

Pooled kwashiorkor serum was studied after it had been loaded with sodium salicylate at various concentrations and subsequently dialysed.

Methods

Dialysis was performed using a new equilibrium dialysis system previously employed by our group.[4] With this

method, dialysis was complete in 30 minutes, but a further 30-minute safety margin was allowed in all cases before dialysis was stopped.

Salicylate estimations were a quantitative modification of Trinder's method,[5] using 0,2 ml of serum.

Statistical analysis involved the use of Student's *t*-test and the coefficient of correlation.[6] Unless otherwise stated, at each salicylate concentration 10 separate dialyses were carried out at a pH of 7,4 - 7,5 at 37°C. In our hands, the semi-micro salicylate assay described above had an error of 3%. Using the dialyser, the error fell from 4%[2] to 2%, because Hamilton syringes were employed for loading and unloading.

Materials

Fresh serum was obtained by venepuncture from the authors, and was pooled and frozen until use.

Fresh plasma. The plasma employed for these studies was fresh frozen plasma (FFP) obtained from the South African Blood Transfusion Service.

Kwashiorkor serum was obtained from children admitted to hospital prior to any form of therapy, to obviate the problem of competitive binding.

RESULTS

Comparison between the Binding Power of Normal Serum and Plasma

The results are depicted in Table I, and shown diagrammatically in Figs 1 and 2. No difference could be found between plasma and serum; indeed, the relationship between the two was very close, as is shown in Fig. 3.

Detailed Study of Plasma Binding

As can be seen from Fig. 1, binding appeared to cease at approximately 40 - 60 mg/100 ml of total salicylate. An attempt was made, using plasma, to find a more precise concentration at which this might occur.

Fig. 4 shows the relationship between bound and total salicylate with 10 different concentrations of the latter. Bound versus free results on the same samples are shown in Fig. 5. Statistical analysis revealed that from 39 mg/100 ml total salicylate upwards, no significant difference in plasma binding could be found. Thus, in normal plasma, binding appeared to cease at about 40 mg/100 ml.

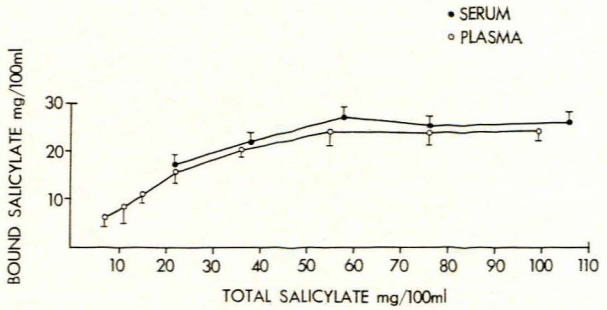

Fig. 1. A diagrammatic representation of the results shown in Table I. Mean plasma and serum bound salicylate concentrations (2 standard deviations) are plotted against the total serum salicylate concentration. No statistical difference ($P>0,2$) was observed between plasma and serum.

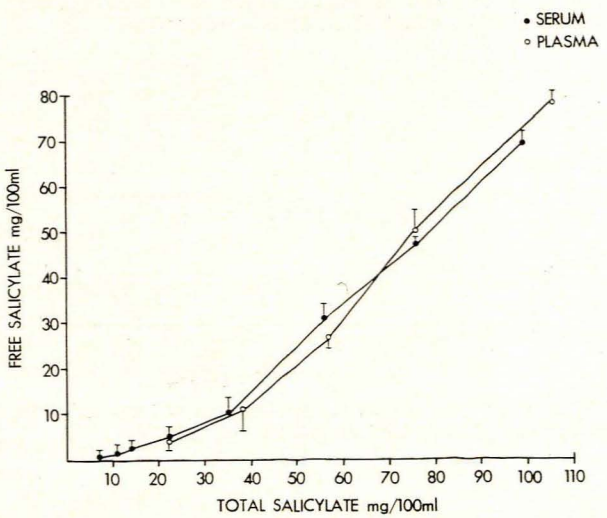

Fig. 2. The mean values and 2 standard deviations of the free salicylate concentration versus total salicylate (a diagrammatic representation of the data in Table I). There was no statistical difference ($P>0,2$) between plasma and serum.

TABLE I. COMPARISON BETWEEN THE BINDING CAPACITY OF PLASMA (ALBUMIN CONCENTRATION 4,2 g/100 ml) AND FRESH SERUM (ALBUMIN CONCENTRATION 4,3 g/100 ml)

	Serum — total salicylate (mg/100 ml)					Plasma — total salicylate (mg/100 ml)								
	27,6	37,7	57,4	76,0	106,7	7,4	10,4	13,4	16,7	22,1	34,9	55,0	75,8	99,3
Number of samples	10	7	10	8	7	7	8	8	7	10	15	10	10	15
Mean bound salicylate (mg/100 ml)	17,4	22,5	27,3	25,0	25,6	6,5	8,7	11,0	12,4	16,3	20,6	24,2	23,5	24,6
Mean free salicylate (mg/100 ml)	4,1	10,4	27,2	49,6	77,4	0	1,4	2,6	2,2	4,3	10,0	30,4	47,2	69,4
% free salicylate (mean)	13,7	30,7	49,7	66,5	75,1	0	14,4	19,1	15,0	20,8	33,2	55,5	66,8	73,8
Mean % of original recovered*	94,6	85,8	94,5	98,5	96,5	87,8	94,3	101,8	87,6	93,2	28,3	98,6	93,0	94,6

* (Bound + free) ÷ original total serum salicylate × 100.

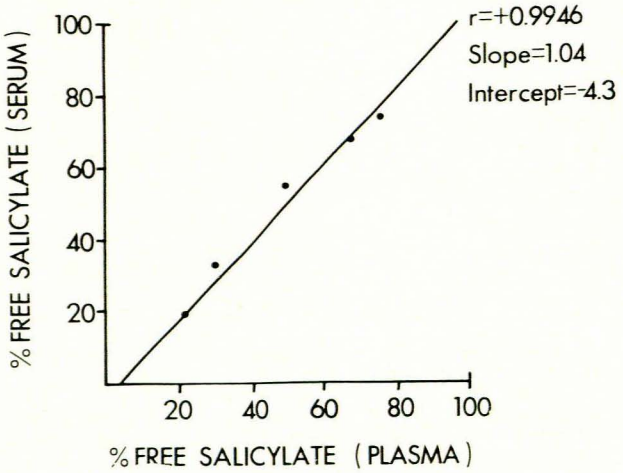

Fig. 3. A close correlation was observed between the percentage of free salicylate in plasma and serum.

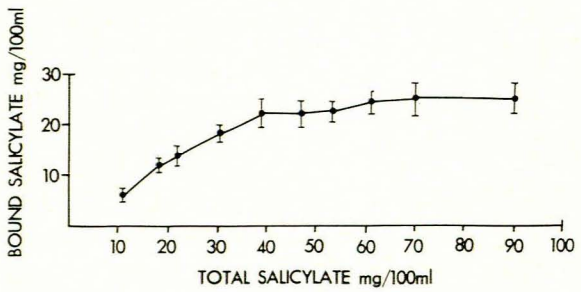

Fig. 4. The relationship between bound and total salicylate in normal plasma (albumin 4,2 g/100 ml).

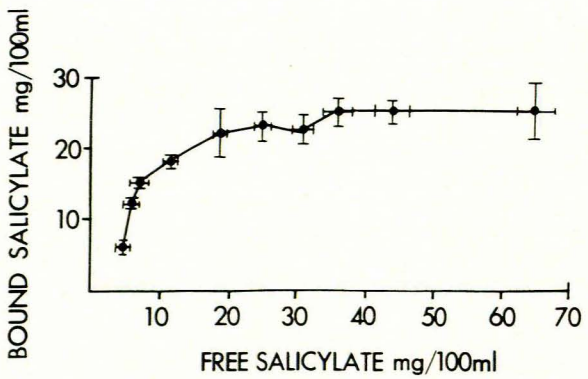

Fig. 5. The relationship between bound and free salicylate in normal plasma (albumin 4,2 g/100 ml).

pH Studies

Three separate pH levels were studied: pH 6,97 (total salicylate 37 mg/100 ml), pH 7,5 (total salicylate 40,5 mg/100 ml) and pH 8,15 (total salicylate 38 mg/100 ml). The results in Table II show a statistically significant difference between the three pH values.

TABLE II. RESULTS OF pH STUDIES AT SIMILAR TOTAL SALICYLATE CONCENTRATIONS*

	Total serum salicylate (mg/100 ml)		
	37	40,5	38
pH	**6,97**	**7,5**	**8,15**
% free salicylate	**36,0 ± 3,5**	**45,9 ± 3,3**	**52,5 ± 2,9**
% bound salicylate	**63,9 ± 3,6**	**54,1 ± 3,2**	**47,4 ± 2,9**

* pH 6,97 differs significantly from pH 7,5 (t = 6,2 N = 18, P = <0,0005), as does pH 7,5 from pH 8,15 (t = 4,4, N = 18, P = <0,0005).

Kwashiorkor Serum versus Normal Plasma

Pooled kwashiorkor serum (albumin 2,3 g/100 ml) was compared with normal plasma (albumin 4,2 g/100 ml). These results are shown in Table III and Fig. 6. The difference from 22 mg/100 ml upwards is highly significant statistically, with kwashiorkor serum showing markedly inferior binding.

TABLE III. COMPARISON BETWEEN THE FREE SALICYLATE CONCENTRATION AND TOTAL SALICYLATE CONCENTRATION IN KWASHIORKOR SERUM AND NORMAL PLASMA

Total salicylate (mg/100 ml)		Free salicylate (mg/100 ml)	
Kwashiorkor	Normal	Kwashiorkor	Normal
15	**12,4**	**4,3 ± 0,6**	**4,8 ± 0,8**
22	**24,2**	**10,6 ± 0,9**	**7,1 ± 1,0**
33,5	**32,2**	**22,8 ± 1,6**	**11,5 ± 0,6**
53	**52,4**	**39,5 ± 1,2**	**26,5 ± 1,6**
80,5	**73,8**	**68,4 ± 0,5**	**44,3 ± 1,5**

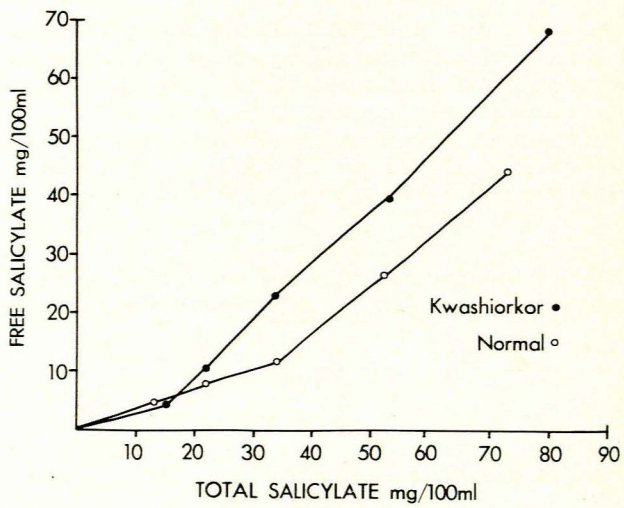

Fig. 6. The difference between kwashiorkor serum and normal plasma. From 22 mg/100 ml upwards, the difference is at less than P = 0,005 level.

DISCUSSION

A variety of methods have been employed in the dialysis of salicylate.[7,8] The advent of a new semi-micro equilibrium dialysis system and a clinical situation worthy of investigation, provided an ideal opportunity to re-evaluate the problem of salicylate binding.

It is known that salicylate binds exclusively to albumin.[9] However, it has been suggested that there might be a difference between the binding capacity of plasma and serum, and that this could be due to either serum globulins or fibrinogen.[3] The observations made in the present study, employing pathophysiological concentrations of salicylate, and after 132 separate dialyses, do not support this contention. The binding capacity of plasma and serum were in no way statistically different. It would thus appear that either plasma or serum can be employed for *in vitro* studies.

Figs 4 and 5 show, and it is confirmed statistically, that salicylate binding ceases at a total salicylate concentration of approximately 40 mg/100 ml. Thereafter there is a progressive accumulation of free salicylate. This figure corresponds well with the generally accepted level of 30 mg/100 ml which is associated with toxicity.[10]

In contradistinction to the report of Moran and Walker,[8] who observed that pH changes in the range 7,0 - 8,0 produced a 3% change in the binding of salicylate at a total serum level of 50 mg/100 ml, the present studies show much greater alterations. However, at above pH 8,0 Moran and Walker observed a greater fall in the bound fraction. Binding was greatest at the lowest pH value studied and decreased progressively as pH rose. A 9,9% decrease in binding was observed over the paediatric pH range (6,97 - 7,5).

This would hold certain advantages for the salicylate-intoxicated infant, who is almost invariably acidotic,[1,2] but would not represent a contra-indication to bicarbonate therapy. In theory, alkalinisation producing a normal pH in these infants would then release about 10% of the bound salicylate; this could be transferred to the CSF, enhancing toxicity, but by virtue of the previously described mechanisms of action of bicarbonate on CSF and brain salicylate,[2,11] this is unlikely to be clinically detrimental.

Perhaps of greatest interest is the observation that there is a marked difference between the binding capacity of kwashiorkor serum and normal plasma. The hallmark of kwashiorkor is hypo-albuminaemia[12] and therefore it is to be expected that drug binding in these infants will be deranged. As far as we know, however, this has not been documented in human malnutrition. Other hypo-albuminaemic situations have been studied in this regard, namely rheumatoid arthritis,[13] chronic infections[9] and the nephrotic syndrome.[14] In a study of salicylate binding to diabetic serum,[15] it was observed that there was a difference between non-diabetic and insulin-dependent diabetic serum: it was felt that this might be related to an alteration in the physicochemical properties of serum albumin in this group of patients. Children with kwashiorkor are, however, known to have qualitatively normal albumin.[16] Moreover, it has been shown[17] that lysine-deficient serum is unable to bind salicylate; although this argument could be used to explain the poor binding observed in kwashiorkor, serum from these patients is not lysine-deficient.[16]

In these studies, binding of salicylate appeared to cease at about 15 mg/100 ml total salicylate when kwashiorkor serum was used. Thereafter, a progressive accumulation of free salicylate occurred. This observation agrees well with the data of Moran and Walker,[8] who carried out a similar study *in vitro*, but without using kwashiorkor serum. If salicylate can be taken as an example of an albumin-bound drug, then the same may apply to many other therapeutic agents. Further studies are in progress.

We wish to thank Professor J. D. L. Hansen for assistance with the manuscript, and the South African Medical Research Council for financial support.

REFERENCES

1. Buchanan, N. and Rabinowitz, L. (1974): J. Pediat., **84**, 391.
2. Buchanan, N., Eyberg, C. and Kunding, H. (1974): *Ibid.* (in press).
3. Stafford, W. L. *in* Dixon, A. St J. ed. (1963): *Salicylates*. London: J. & A. Churchill.
4. Buchanan, N. and Eyberg, C. (1974): S. Afr. Med. J., **48**, 1867.
5. Trinder, R. (1954): Biochem. J., **57**, 301.
6. Bradford Hill, A. (1971): *Principles of Medical Statistics*. London: Lancet.
7. Stafford, W. L. (1962): Biochem. Pharmacol., **11**, 685.
8. Moran, C. J. and Walker, W. H. C. (1968): *Ibid.*, **17**, 153.
9. Reynolds, R. C. and Cluff, L. E. (1960): Bull. Johns Hopk. Hosp., **107**, 278.
10. Harrison, T. R. ed. (1966): *Principles of Internal Medicine*. New York: McGraw-Hill.
11. Hill, J. B. (1971): Pediatrics, **47**, 658.
12. Whitehead, R. G., Coward, W. A. and Lunn, P. G. (1973): Lancet, **1**, 63.
13. Fremont Smith, K. *in* Dixon, A. St J. ed. (1963): *Op. cit.*[3]
14. Lewis, G. P., Jusko, W. J. and Burke, C. W. (1971): Lancet, **2**, 778.
15. Stafford, W. L. (1962): *Ibid.*, **1**, 243.
16. Potgieter, G. M., Hiner, M. and Kench, J. E. (1967): Clin. chim. Acta, **18**, 107.
17. Davison, C., Spitzer, L. E. and Smith, P. K. (1959): Fed. Proc., **18**, 381.