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Pharmacokinetics

Evaluation of existing limited sampling models for busulfan kinetics in children with beta thalassaemia major undergoing bone marrow transplantation

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Summary:

Busulfan pharmacokinetic parameters are useful in predicting the outcome of allogeneic bone marrow transplantation (BMT). Standard pharmacokinetic measurements require multiple blood samples. Various limited sampling models (LSM) have been proposed for reducing the sample number required for these measurements, essentially for patients with malignant disorders undergoing BMT. This study was undertaken to evaluate the existing LSM for busulfan pharmacokinetics to find out the most suitable method for patients with thalassaemia major undergoing BMT. Busulfan levels in plasma samples were analysed by HPLC. The AUC calculated by non-compartmental analysis using the program 'TOPFIT' was compared with previously published LSMs. Our seven sample pharmacokinetic data for AUC calculation was compared with the published LSMs. The three sample models suggested by Chattergoon *et al* and Schuler *et al* showed significant agreement with AUC TOPFIT ($R^2 = 0.98$ and 0.94, respectively) in our clinical context. Other models resulted in significant over or under representation of observed values (Vassal's model $R^2 = 0.61$; Chattergoon's two sample model $R^2 = 0.84$; four sample model $R^2 = 0.83$; Schuler's two sample model $R^2 = 0.79$). By these data the three sample LSM proposed by Chattergoon *et al* and Schuler *et al* are suitable for calculation of the AUC in patients with thalassaemia major undergoing BMT conditioned with oral busulfan. *Bone Marrow Transplantation* (2001) **28,** 821–825.

Keywords: busulfan; limited sampling model; thalassaemia; bone marrow transplantation

Busulfan, a bifunctional alkylating agent of the methyl sulfonate group, in combination with cyclophosphamide is widely employed in conditioning regimens for bone marrow transplantation (BMT),[1–3] A large number of studies has shown that several factors including age of the patient,[4–6] underlying disease,[7,8] chronopharmacology,[9,10] food[11] and concomitant administration of other drugs[12] affect the pharmacokinetics of oral busulfan. It has also been demonstrated that both in adult and pediatric patients, dose adjustment according to pharmacokinetic parameters could improve the outcome of allogeneic BMT by reducing regimen-related toxicities and relapse of disease.[13,14] Most patients with thalassaemia major undergoing BMT have abnormal liver functions due to iron overload and hepatitis virus infections.[15] A high incidence of hepatic toxicities related to conditioning therapy has been reported.[16] We have also shown that there is a correlation between busulfan pharmacokinetics and rejection in these patients.[17] Evaluation of busulfan pharmacokinetics therefore acquires particular significance in these patients.

The conventional seven to 12 sample (per dose) model is accurate for analysis of busulfan kinetics but requires frequent blood sampling, which is inconvenient for the patient, nursing and laboratory staff, and increases the cost of evaluation. Various studies have proposed limited sampling models (LSM) for determination of AUC based on two to three samples per dose of busulfan. These LSM approaches provided data comparable to those of conventional sampling procedures but without the disadvantages of the latter. However, such correlations have essentially been assessed in patients with malignant disorders undergoing BMT. So far, LSMs have not been evaluated in children with non-malignant inherited disorders. The purpose of this study was to explore whether a LSM was applicable to patients with thalassaemia major undergoing BMT. To this end, we have compared the busulfan AUC (calculated by TOPFIT) obtained from conventional multisampling procedure to that obtained by LSM procedures in order to establish the most suitable method for patients with thalassaemia major.

Patients and methods

Patients

Patients with beta thalassaemia major undergoing BMT were assigned to one of the two conditioning regimens as

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previously described:[18] regimen A: busulfan 16 mg/kg + ALG + cyclophosphamide 200 mg/kg and regimen B: busulfan 600 mg/m^2 + cyclophosphamide 200 mg/kg. Blood samples from children with thalassaemia were collected in heparinized tubes before each dose and after 0.5, 1, 1.5, 2, 4 and 6 h after doses 1, 2 and 13. Informed consent was obtained from the patients' parents and ethical approval was obtained from the Institutional Review Board.

Pharmacokinetic analysis

Busulfan in plasma samples was analysed by HPLC as previously described.[19] AUC was calculated by non-compartmental analysis using the computerized program TOPFIT (version 2.0).[20]

Limited sampling models

The AUC in the published LSM are calculated by using a combination of the trapezoidal rule, which is used to calculate AUC from time 0 to a particular dosing interval (eg 0–6 h) and the logarithmic rule, which derives the extrapolated AUC up to infinity using the formula Cx/Ke, where Cx = plasma concentration at time x after the dose and Ke is the elimination rate constant. These formulae were arrived at by stepwise multiple linear regression with the AUC as dependent and the individual concentrations as independent variables. Busulfan AUC calculated by TOPFIT was compared with AUCs calculated using the following LSMs:

(1) Chattergoon *et al*:[21]
- two sample: $AUC = 30C_{1h} + 300C_{1h}/(Ln\ C_{1h} - LnC_{6h})$
- three sample: $AUC = 45C_{1h} + 15C_{1.5h} + 270C_{1.5h}/(Ln\ C_{1.5h} - Ln\ C_{6h})$
- four sample: $AUC = 45C_{1h} + 30C_{1.5h} + 15C_{2h} + 270C_{2h}/(Ln\ C_{2h} - Ln\ C_{6h})$

(2) Vassal *et al*:[22] $AUC = 122 + 0.97C_{0.5h} + 13.94C_{6h}$

(3) Schuler *et al*:[11]
- two sample: $AUC = 782 + 1.42C_{1h} + 3.74C_{4h}$
- three sample: $AUC = 289 + 1.16C_{1h} + 1.06C_{2h} + 3.16C_{4h}$

where $C_{0.5h}$, C_{1h}, $C_{1.5h}$, C_{2h}, C_{4h} and C_{6h} represents the busulfan plasma concentrations after 0.5, 1, 1.5, 2, 4 and 6 h, respectively, of busulfan dose; Ln represents the natural logarithm. Chattergoon's and Vassal's models were proposed for children and estimate AUC 0–inf whereas Schuler's model was proposed for adults and estimates AUC 0–6 h.

Statistical analysis

Linear regression analysis was applied to compare the AUCs calculated by TOPFIT *vs* AUC calculated by various LSMs, using SPSS version 7.5 for Windows.

Results and discussion

The estimated AUC values derived from different LSM protocols were compared with AUC values obtained from conventional measurement of all samples using TOPFIT[20] (Table 1). When analyzed by linear regression analysis, a significant agreement ($R^2 = 0.79–0.98$) was found between all these models except with the model proposed by Vassal *et al*[22] ($R^2 = 0.61$, Table 1). The mean difference in the calculated AUC for each of these models from the observed values was less than 5% for all except the two sample model of Chattergoon *et al*[21] and the model proposed by Vassal *et al*[22] where it was lower by 5.29% and 6.38%, respectively. Figure 1 describes the linear regression of AUCs calculated by all the proposed LSMs *vs* TOPFIT. Figure 2 shows the ratio plots of AUCs estimated by these models and those determined by TOPFIT.

The best correlation was found between AUC TOPFIT and the three sample LSM of Chattergoon *et al*[21] ($R^2 = 0.98$). Although all other models except the one proposed by Vassal *et al*[22] correlated significantly, the mean difference was much lower with this model (2.35%, range 1.6–5.5%). AUC (0–6 h) calculated by Schuler's[11] three sample LSM also showed a good agreement ($R^2 = 0.94$) with AUC (0–6 h) TOPFIT, with a mean difference of 1.38% (range 0.6–2.5%), although this model was proposed for adults. However, Hassan *et al*[23] have reported that this model of Schuler *et al*'s[11] resulted in a mean underestimation of 25% (range 2–50%) in the calculation of AUC in children and that the differences were more pronounced at higher AUCs. It was their conclusion that Schuler's model was proposed for AUC calculation in adults and it therefore cannot be used to estimate AUC in children. A similar discrepancy was observed by Chattergoon *et al*[21] when compared with Schuler's LSM. Schuler's AUC calculations were based on AUC 0–6 h at first dose, whereas Chattergoon and Hassan calculated AUC 0–inf. It is remarkable that our data from children with thalassaemia major correlated well with Schuler's three sample LSM in adults. The reason for this is not clear but suggests that the cause of discordance noted by Hassan and Chattergoon may not be the age difference of the subjects studied. Schuler's two sample model showed a less significant agreement with AUC (0–6 h) TOPFIT than the three sample model ($R^2 = 0.79$).

Vassal's[22] LSM was proposed for children in the age range of 1.92 to 13.83 years. When we applied it for calculating AUC for our patient group, we noted significant differences when compared to the AUC obtained by conventional multisample procedure using TOPFIT, with a mean difference of 6.4% (range 1–26%). This discrepancy may be attributed to variation in T_{max} observed in the present study (0.5–6 h). The median T_{max} in the present study was 1.5–2 h and Vassal's[22] model does not use 1.5h or 2h value for AUC calculation. This might explain the observed correlation with other LSMs (all of which include a 1.5 h or 2 h value) with TOPFIT and not with Vassal's[22] model. Previously it was reported[21,23] that Vassal's[22] LSM gave closer results to the determined values, but with a higher degree of variation and a tendency for overestimation at higher AUCs, which was most probably due to

Table 1 Comparison of AUC TOPFIT with published LSM

	AUC (0–inf) TOPFIT	*Chattergoon's LSM*			*Vassal's LSM*	*Schuler's LSM*	
		2 sample	*3 sample*	*4 sample*		*2 sample*	*3 sample*
Mean AUC ± s.d.	3526 ± 855	3054 ± 787	3411 ± 710	3207 ± 963	3577 ± 1212	2898 ± 779	3249 ± 765
CV (%)	—	10	4	9	13	12	3.3
R^2 value	—	0.87	0.97	0.83	0.61	0.89	0.94
Mean % diff	—	5.29	2.35	3.19	6.38	3.69	1.38

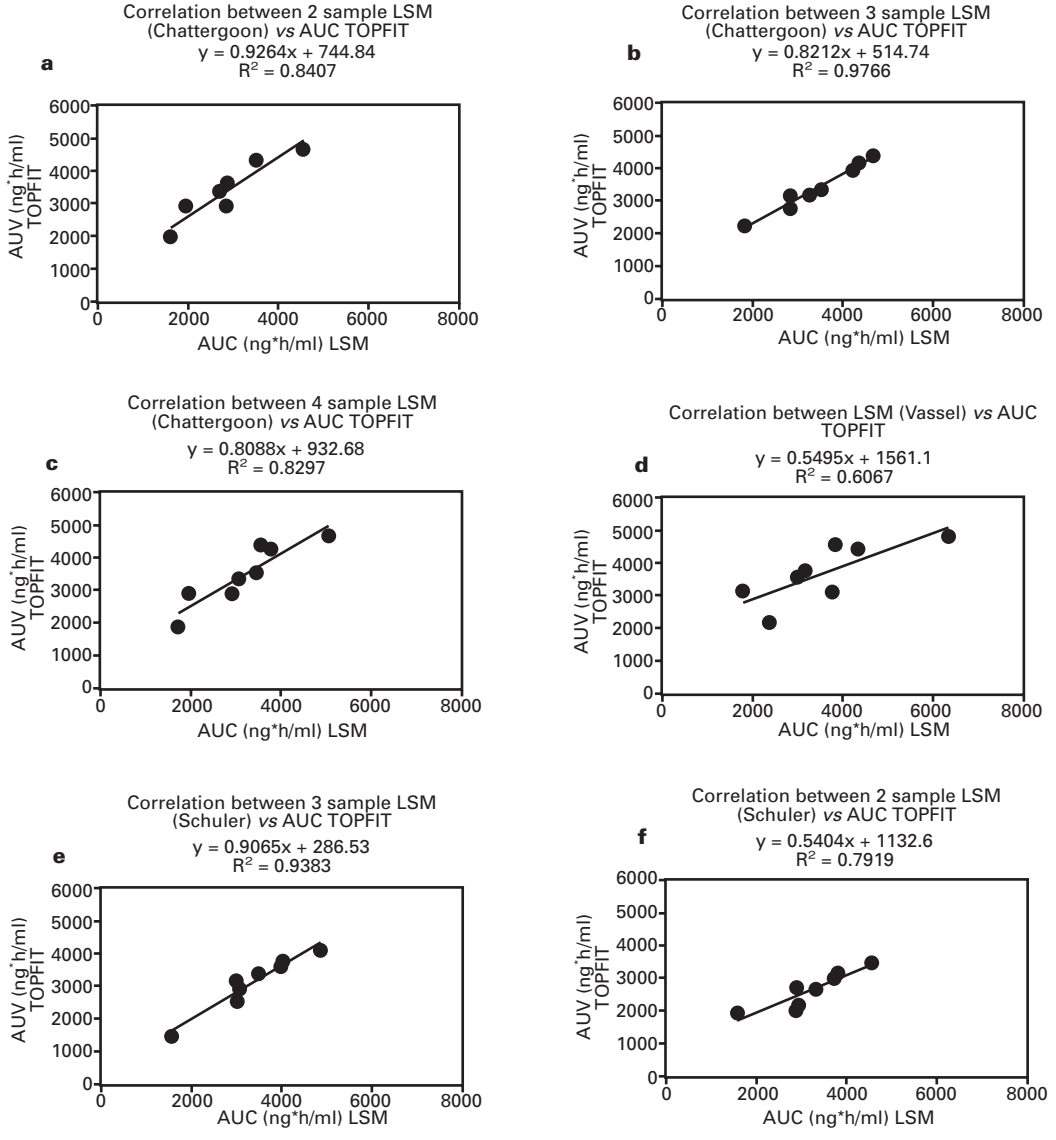

Figure 1 Comparison of AUC TOPFIT with different LSMs. (**a**–**f**) Linear regression curves for AUC TOPFIT *vs* AUCs determined by different LSMs: (**a**, **b** and **c**) correlation between AUC TOPFIT and the determined AUCs calculated using (**a**) two, (**b**) three, and (**c**) four sample LSMs of Chattergoon *et al*;[21] (**d**) correlation between AUC TOPFIT and the determined AUCs calculated using Vassal's LSM;[22] (**e** and **f**) correlation between AUC TOPFIT and the determined AUCs calculated using two and three sample LSM of Schuler *et al*.[11]

wider variation in concentration at 0.5h after administration. We could not make a comparison with Hassan's[23] model because there was no 3 h sample in the present study.

Of all these models, Chattergoon's[21] three sample model and Schuler's[11] three sample model showed the maximum agreement with our AUC determined by TOPFIT. Similarly, Chattergoon *et al*[21] showed the highest agreement between their three sample model and the AUC determined by the KINFIT program.[24] Although other authors have compared the published LSMs for AUC calculations with

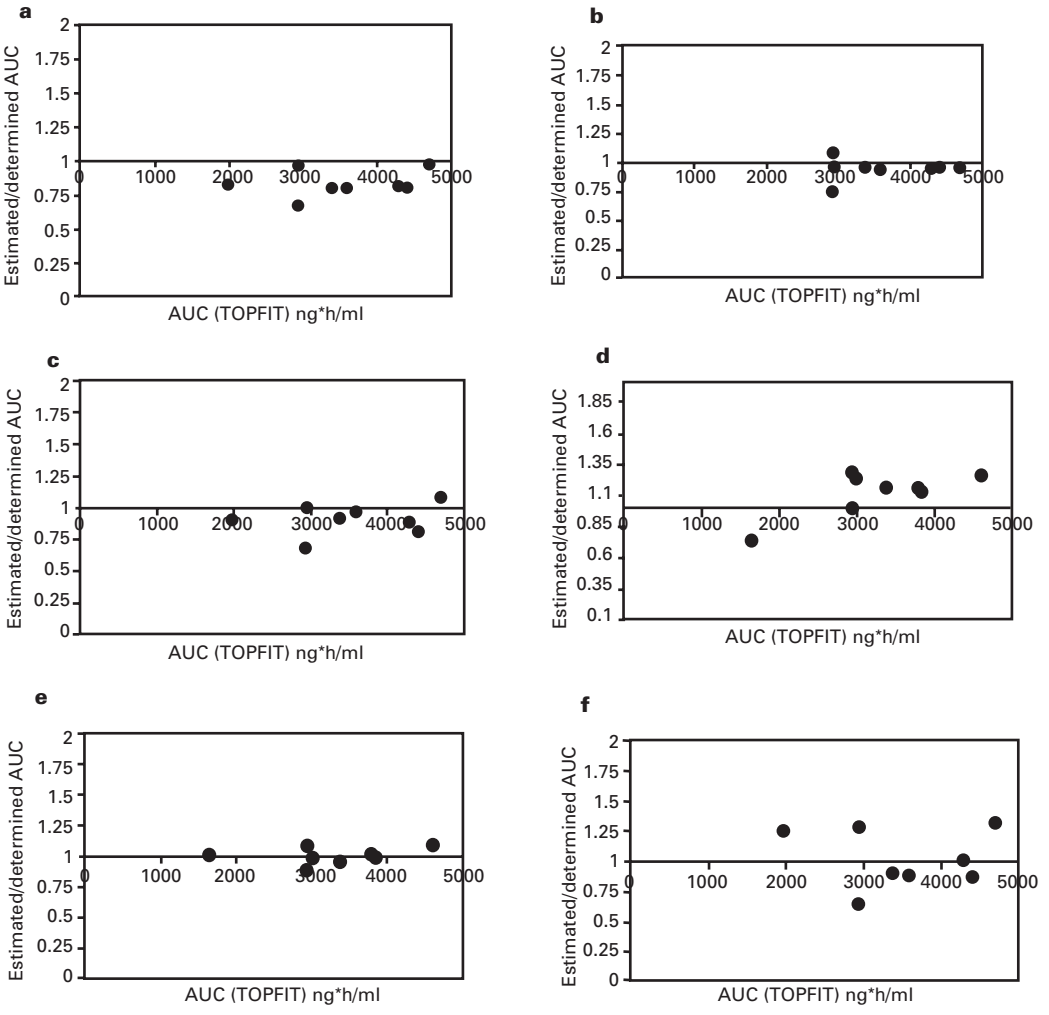

Figure 2 Ratio plots of AUC estimated by different LSM *vs* AUC estimated by TOPFIT. (**a**–**f**) Ratio plots made by plotting AUC TOPFIT (ng*h/ml) *vs* the ratio of AUC estimated by TOPFIT/AUC determined by different LSMs: (**a**, **b** and **c**) Relationship between AUC determined by TOPFIT and estimated/determined AUCs for (**a**) two sample, (**b**) three sample and (**c**) four sample LSM of Chattergoon *et al*;[21] (**d** and **e**) Relationship between AUC determined by TOPFIT and estimated/determined AUCs for (**d**) two sample and (**e**) three sample LSM of Schuler *et al*;[11] (**f**) Relationship between AUC determined by TOPFIT and estimated/determined AUCs for LSM of Vassal *et al*.[22]

their models,[21,23] none of the authors reported significant correlation between the models. The correlation of determined AUC observed in the present study with other models could be due to the use of the non-compartmental model for AUC calculation in this study, as opposed to the one compartment models used by Hassan[22] and Chattergoon.[21]

In conclusion, we have identified Chattergoon's[21] (using 1 h, 1.5 h and 6 h samples) and Schuler's (using 1, 2 and 4 h samples) three sample models to be the most suitable for AUC calculation in children with thalassaemia major for busulfan dose adjustment. These data can now be used to apply LSM for assessment of busulfan pharmacokinetics in patients with thalassaemia major undergoing BMT and thus contribute to cost reduction, and convenience in sample analysis and above all patient's comfort.

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References

1 Santos GW, Tutschka PJ, Brookmeyer R *et al*. Marrow transplantation for acute non-lymphocytic leukaemia after treatment with busulfan and cyclophosphamide. *New Engl J Med* 1983; **309**: 1347–1353.
2 Hobbs JR, Hugh Jones K, Shaw PJ *et al*. Engraftment rates related to busulfan/cyclophosphamide dosages for displacement marrow transplantation in 50 children. *Bone Marrow Transplant* 1986; **1**: 201–208.
3 Lucarelli G, Galimberti M, Polchi P *et al*. Bone marrow transplantation in patients with thalassemia. *New Engl J Med* 1990; **322**: 417–421.
4 Slattery JT, Sanders JE, Buckner CD *et al*. Graft rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. *Bone Marrow Transplant* 1995; **16**: 31–42.
5 Grochow LB, Jones RJ, Brundrett RB *et al*. Pharmacokinetics

of busulfan: correlation with veno-occlusive disease in patients undergoing bone marrow transplantation. *Cancer Chemother Pharmacol* 1989; **25**: 55–61.
6 Regazzi MB, Locatelli F, Buggia I *et al*. Disposition of high dose busulfan in pediatric patients undergoing bone marrow transplantation. *Clin Pharmacol Ther* 1993; **54**: 45–52.
7 Vassal G, Fischer A, Challine D *et al*. Busulfan disposition below the age of three: alteration in children with lysosomal storage disease. *Blood* 1993; **82**: 1030–1034.
8 Gibbs JP, Gooley T, Corneau B *et al*. The impact of obesity and disease on busulfan oral clearance in adults. *Blood* 1999; **93**: 4436–4440.
9 Hassan M, Oberg G, Bekassy AN *et al*. Pharmacokinetics of high dose busulfan in relation to age and chronopharmacology. *Cancer Chemother Pharmacol* 1989; **28**: 130–134.
10 Vassal G, Challine D, Koscielny S *et al*. Chronopharmacology of high dose busulfan in children. *Cancer Res* 1993; **53**: 1534–1537.
11 Schuler U, Schroer S, Kuhnle A *et al*. Busulfan pharmacokinetics in bone marrow transplant patients: is therapeutic drug monitoring warranted? *Bone Marrow Transplant* 1994; **14**: 759–765.
12 Hassan M, Oberg G, Bjorkholm M *et al*. Influence of prophylactic anticonvulsant therapy on high dose busulfan kinetics. *Cancer Chemother Pharmacol* 1993; **33**: 181–186.
13 Dix SP, Wingard JR, Mullins RE *et al*. Association of busulfan area under the curve with veno-occlusive disease following BMT. *Bone Marrow Transplant* 1996; **17**: 225–230.
14 Demirer T, Buckner CD, Appelbaum FR *et al*. Busulfan, cyclophosphamide and fractionated total body irradiation for allogeneic marrow transplantation in advanced acute and chronic myelogenous leukaemia: phase I dose escalation of busulfan based on targeted plasma levels. *Bone Marrow Transplant* 1996; **17**: 341–346.
15 Olivieri N. Thalassaemia: clinical management. *Baillières Clin Haematol* 1998; **11**: 147–162.
16 Richardson P, Guinan E. The pathology, diagnosis and treatment of hepatic veno-occlusive disease: current status and novel approaches. *Br J Haematol* 1999; **107**: 485–493.
17 Dennison D, Chandy M, Poonkuzhali B *et al*. Plasma busulfan levels influence rejection in bone marrow transplantation for homozygous beta thalassaemia. *Blood* 1998; **92**: 127a.
18 Poonkuzhali B, Srivastava A, Quernin MH *et al*. Pharmacokinetics of oral busulfan in children with thalassemia undergoing BMT. *Bone Marrow Transplant* 1999; **24**: 5–11.
19 Quernin MH, Poonkuzhali B, Medard Y *et al*. High performance liquid chromatographic method for quantification of busulfan in plasma after derivatization by tetrafluorothiophenol. *J Chromatogr* 1999; **721**: 147–152.
20 Heinzel G, Woloszezak R, Thoman P. *Pharmacokinetic pharmacodynamic data analysis system for the PC*. Gustav Fischer: Stuttgart, Germany, 1993.
21 Chattergoon DS, Saunders EF, Klein J *et al*. An improved limited sampling method for individualized busulfan dosing in bone marrow transplantation in children. *Bone Marrow Transplant* 1997; **20**: 347–354.
22 Vassal G, Deroussent A, Challine D *et al*. Is 600 mg/m^2 the appropriate dosage of busulfan in children undergoing bone marrow transplantation? *Blood* 1992; **79**: 2475–2479.
23 Hassan M, Fasth A, Gerritsen B *et al*. Busulfan kinetics and limited sampling model in children with leukaemia and inherited disorders. *Bone Marrow Transplant* 1996; **18**: 843–850.
24 D'Argenio DZ, Schumitzky A. A program package for simulation and parameter estimation in pharmacokinetic systems. *Comp Prog Biomed* 1979; **9**: 115–134.