Diskussion

Die vorliegenden Untersuchungsergebnisse haben gezeigt, daß die Blut-Liquorschranke nicht nur für 5-Carbamyl-5H-dibenzo[b, f]azepin, sondern auch für die zwei beschriebenen Metaboliten durchgängig sein muß. Auf Grund von Beobachtungen, wie sie sich bei der qualitativen dünnschichtchromatographischen Untersuchung anderer Körperflüssigkeiten, wie Serum, Harn und Galle ergaben, muß angenommen werden, daß die beiden Metaboliten hier ebenfalls vorkommen. Hierüber, sowie über die Pharmakodynamik des Tegretal, d. h. über seine Konzentration in Blut, Galle, Liquor und Harn in Abhängigkeit von der Art und dem Zeitpunkt der Applikation, wird ausführlich an anderer Stelle berichtet (1).

Die relativ einfache Methodik und insbesondere ihre große Empfindlichkeit erlauben außerdem, die Verteilung des Tegretal in Organen, z. B. auch im Hirn, ohne Anwendung der Isotopentechnik zu bestimmen. Inwieweit allerdings die zentralen Effekte des Tegretal allein auf die Substanz oder auf einen der entstandenen Metaboliten zurückzuführen sind, muß vorerst noch offen bleiben. Die gute Liquorgängigkeit, sowie die Affinität zum Hirngewebe und der günstige therapeutische Effekt, insbesondere bei Erkrankungen mit Quellungsprozessen bzw. lokalen Ödemreaktionen im Gehirn, legen die Vermutung nahe, daß Tegretal einen Permeabilitätseffekt in diesem Bereich entfaltet. Allerdings besitzt diese Annahme nur den Wert einer Arbeitshypothese. Ihre Gültigkeit bleibt zu beweisen.

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Continuous "in vivo" registration of bromsulfalein disappearance curves

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The reliability of the automated continuous "in vivo" registration of BSP with AutoAnalyzer is tested. Impairment of dialysis occurs when 0,80 ml/min blood is aspirated as recommended by other authors. When a BSP solution with constant concentration in blood, plasma or serum is registered making use of this manifold, a progressive decrease of extinction is obtained and therefore the calculated concentration becomes falsely low. It is not impossible that the same phenomenon can be found with technics for other continuous registrations when relatively great amounts of blood are aspirated. The performed experiments prove the necessity of using a tube with small delivery (e. g. 0,32 or 0,16 ml/min) for aspiration of sample.

Die Zuverlässigkeit des Verfahrens der automatischen Dauerregistrierung von BSP „in vivo" mit dem Autoanalyzer wird getestet. Eine Verschlechterung der Dialyse entsteht, wenn 0,8 ml/Min. Blut aspiriert wird, wie andere Autoren es empfehlen. Wenn eine BSP-Lösung mit konstanter Konzentration in Blut, Plasma oder Serum registriert wird mit diesem Fließ-Schema, wird eine progressive Abnahme der Extinktion erhalten, und deshalb wird die Konzentration fälschlich zu niedrig bewertet. Es ist nicht unmöglich, daß das gleiche Phänomen auch bei Techniken für andere fortlaufende Registrierungen gefunden werden kann, wenn verhältnismäßig große Blutmengen aspiriert werden. Die ausgeführten Versuche beweisen die Notwendigkeit, Leitungen zu verwenden, die kleine Mengen (z. B. 0,32 oder 0,16 ml/Min.) für die Aspiration der Einzelprobe liefern.

The automated continuous "in vivo" registration of bromsulfalein ("BSP") seems a promising method for investigation of hepatic function in health and disease. The object of the present study was to analyse some factors which can interfere with the reliability of the method.

Methods and results

We made use of the AutoAnalyzer manufactured by Technicon Instruments Corporation. The flow diagram is represented in figure 1. A flow cuvette of 15 mm light path being available, a range expander and a double set of dialysis plates (1) seemed unnecessary when 0,80 ml/min. sample is aspirated. The former was only used when the sample flow was reduced to 0,32 or 0,16 ml/min.

Two different manifolds (one being the unchanged manifold according to Galli et al. [1]) — having in common an identical sample-tube with delivery 0,80 ml/min. but differing by the flow of alkaline reagent[1]) and

[1]) *Alkaline reagent:* sodium hydroxide 2,0 g; sodium chloride 7,0 g; sodium p-toluenesulfonate 6,4 g; twice-distilled water 1000 ml.

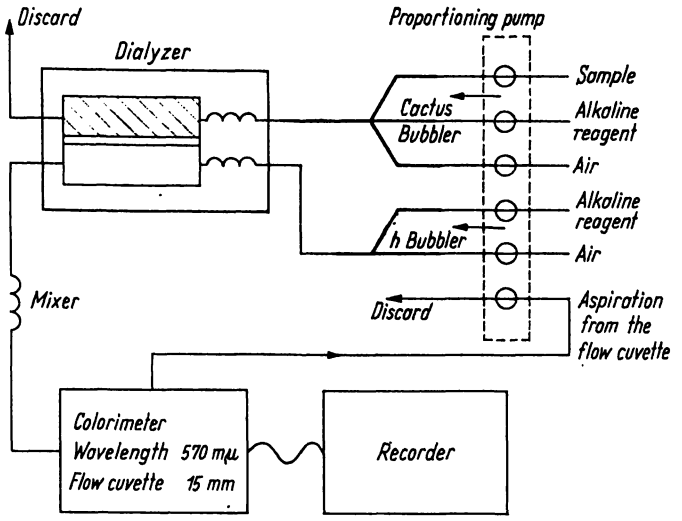

Fig. 1
Flow diagram for continuous registration of BSP disappearance curves.

air (tab. 1) — were used for continuous "in vivo" registration. When two successive doses of BSP (5 mg/kg body weight) are injected intravenously into the same patient with an interval of 45 minutes, we registered — using both manifolds — smaller initial extinction values for the second curve (fig. 2a) whereas the BSP plasma concentrations determined with a spectrophotometer (wavelenght 580 mμ) were higher (fig. 2b).
This observation incited us to the following experiments. BSP solutions with constant concentration in twice-

Tab. 1
Continuous registration of a constant BSP solution

A. Unchanged manifold according to GALLI et al. (1)	Tube I. D.	Delivery (m/min)
Sample	0,045″	0,80
Alkaline reagent	0,045″	0,80
Air	0,073″	2,00
Alkaline reagent	0,065″	1,60
Air	0,073″	2,00

100 mg ‰ BSP in:	Concentration (mg‰) calculated from the obtained extinction. After 30 min.	After 60 min.
- whole blood	77	55,2
- plasma	76	52
- serum	74,6	57,3

B. Manifold	Tube I. D.	Delivery (m/min.)
Sample	0,045″	0,80
Alkaline reagent	0,035″	0,42
Air	0,056″	1,20
Alkaline reagent	0,056″	1,20
Air	0,056″	1,20

100 mg ‰ BSP in:	Concentration (mg ‰) calculated from the obtained extinction After 30 min.	After 60 min.
- aqua bidist.	100	100
- blood diluted I/5 in phys. saline	98	96
- whole blood	78	65
- plasma	79	60
- serum	76	53

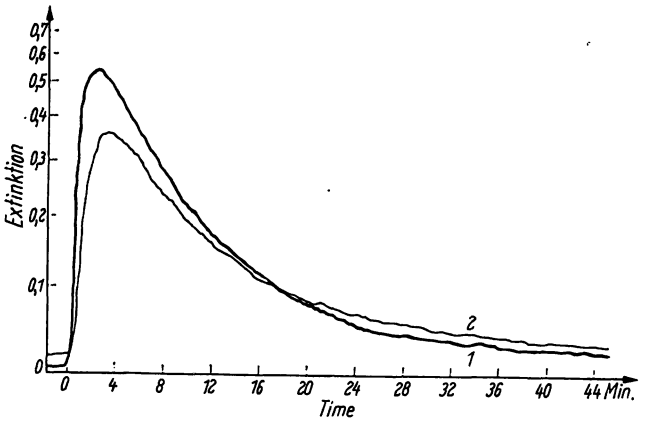

Fig. 2a
Superimposed recordings of two successive disappearance curves (1 and 2) in the same patient (5 mg/kg body weight BSP, injected intravenously with an interval of 45 minutes). The initial extinction values of the second curve are smaller if a sample-tube of 0,80 m/l min. delivery is used.

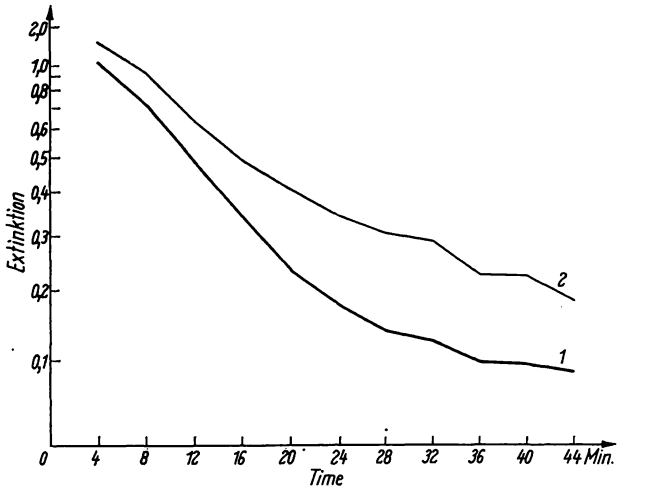

Fig. 2b
Plasma concentrations measured spectrophotometrically on samples, taken every 4 minutes during the first and second curve drawn in figure 2a.

distilled water, in blood diluted with physiologic saline, in whole blood, plasma or serum are recorded during one hour, using both manifolds mentioned above. The results are presented in table 1. Although the BSP concentration is constant, a marked and progressive *decrease of extinction* — and thus of calculated concentration — is obtained when 0,80 m/min. whole blood, plasma or serum is aspirated. The decline of extinction is greatly prevented when the amount of aspirated blood has diminished by previous dilution with physiologic saline.
These experiments were controlled with 3 different colorimeters and comparable results were obtained. An unstable colorimeter can theoretically simulate a similar diminution of the extinction. However, in that eventuality, the base-line wouldn't be stable, the registration of a BSP solution in water would give a comparable decline and the decline would continue when stopping the proportioning pump so the same dialysate stagnates in the flow cuvette.
The decline of extinction obtained by using a manifold with a sample-tube of great delivery (0,80 m/min.

whole blood, plasma or serum) apparently is caused by impairment of dialysis through the Cuprophan membrane as is proved by the following tests:

a. The spectrophotometrical determination of the BSP concentration shows a progressive decrease in the dialysate comparable with the results obtained on the recorder of the AutoAnalyzer, while the BSP concentration in the contradialysate increases.

b. When an aqueous BSP solution is registered immediately after a BSP solution in plasma, serum or blood, the extinction progressively increases until an extinction corresponding with the concentration of the dye is obtained (re-establishment of normal permeability of the membrane). This normalisation of permeability is accelerated by cleaning with a detergent solution.

The reduction of the amount of aspirated whole blood, plasma or serum to 0,32 ml/min. reduces the decline of extinction (tab. 2) as does the dilution of whole blood with physiologic saline when using the previous manifolds with sample flow of 0,80 ml/min. Figure 3, which represents again the superimposed disappearance curves of two successive intravenous doses of BSP injected into the same patient with an interval of 45 minutes, shows that a manifold with sample-tube of 0,32 ml/min. delivery also gives more reliable results "in vivo". — Using a sample-tube of 0,16 ml/min. delivery, no decline of extinction was obtained during a registration for one hour of a BSP solution in plasma.

Tab. 2
Continuous registration of a constant BSP solution

Manifold	Tube I. D.	Delivery (ml/min.)
Sample	0,030″	0,32
Alkaline reagent	0,073″	2,00
Air	0,045″	0,80
Alkaline reagent	0,073″	2,00
Air	0,056″	1,20

100 mg ‰ BSP in:	Concentration (mg ‰) calculated from the obtained extinction	
	After 30 min.	After 60 min.
- aqua bidist.	100	100
- whole blood	98	95,5
- serum	99,5	99
- plasma	97	95

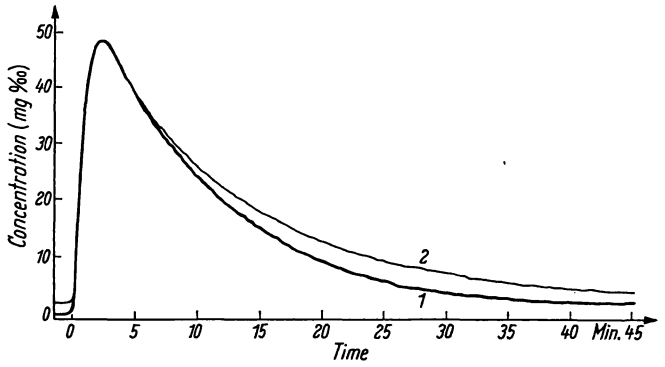

Fig. 3a

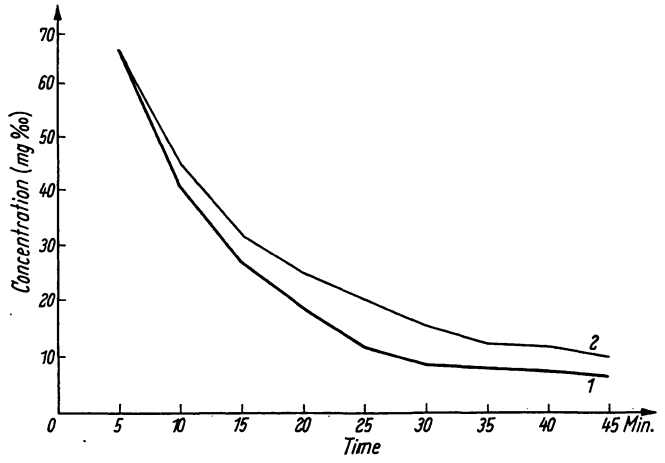

Fig. 3b

Fig. 3

Superimposed recordings of two successive BSP disappearance curves (fig. 3a) — making use of sample-tube with 0,32 ml/min. delivery — compared with BSP plasma concentrations, determined on samples taken every 5 minutes during these curves (fig. 3b).

Comments

Our experiments clearly prove the necessity of using a manifold with a tube of small delivery for aspiration of sample — e. g. 0,32 or even better 0,16 ml/min. In this way, the use of a range expander cannot be avoided even with a flow cuvette of 15 mm light path.

When a BSP disappearance curve is registered with the manifold described by GALLI et al. (1) it may be expected that the fractional clearance (K) obtained will be greater than the real one as determined spectrophotometrically on plasma samples. The impairment of dialysis will falsify the results even more when a registration with this manifold is performed during a continuous infusion of BSP (3) in order to calculate the relative storage capacity (S) and excretory transport maximum (Tm). — Other methods for continuous "in vivo" registrations certainly must be controlled. It is not impossible that the same phenomenon of impaired dialysis occurs when a too large amount of blood is aspirated.

The nature of the substance in blood, plasma or serum which causes the impairment of dialysis cannot be identified with the performed experiments. The sodium para-toluenesulfonate (2) added to the alkaline reagent, is not responsible because a similar diminution of extinction is obtained with or without this product.

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