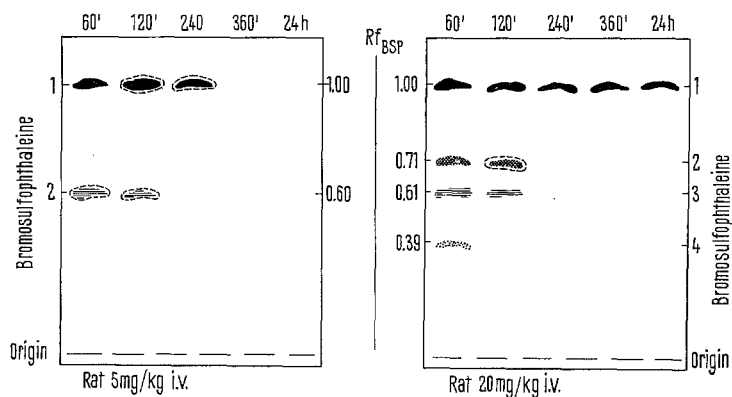


## Urinary Metabolites of Bromosulphophthaleine in Normal Rat

Since significant data are not found in the literature about the urinary elimination of bromosulphophthaleine (BSP) in normal rat<sup>1-4</sup>, we found it interesting to resume this argument, using a new chromatographic method that is quite sensitive for this dye and its metabolites.

**Materials and method.** 20 normal Wistar rats, of both sexes (mean weight  $230 \pm 20$  g), were fasted 12 h before the experiment. 5 mg/kg of BSP were injected i.v. in 10 rats and 20 mg/kg in the other 10 rats. Just before the injection of BSP the rats were hydrated by 25 ml/kg of physiological solution. The urine samples were taken away from the collecting vessels at 60, 120, 240, 360 min and 24 h from the injection of BSP. The whole urine of each sample was dried in a rotating evaporator (under vacuum) at a temperature not above 40°C. The dry residues were then added with 1 or 2 ml of water/acetone

obtained. The higher dose (20 mg/kg) can prolong considerably the dye elimination (as long as 24 h); moreover the appearance of metabolites not visible with the dose of 5 mg/kg of BSP can be noted. Leaving out of consideration the origin and nature of such metabolites, it appears that situations very different have to be interfered in the elimination of BSP into urine, even if the hepatocyte function is normal. The fact that only by the increase of BSP dose the appearance of a greater number of urinary metabolites than 5 mg/kg, opens new perspectives about the action not only of the liver but also of other organs (especially the kidney) by the elimination of exogenous substances from the body for diagnostic and therapeutic purposes. Preliminary trials, made in man using the same doses of BSP, have given similar and characteristic results for each of the 2 doses<sup>5,6</sup>.



Chromatography of normal rat's urine after i.v. injection of bromosulphophthaleine.

(1:1) and centrifuged. The plates ( $20 \times 40$  cm, 0.5 mm thick, automatic stratifier) were prepared with Kieselgel GF<sub>254</sub> and distilled water (1:2). The plates were dried in air for not less than 24 h and activated at 110°C for 30 min. As an eluent tertiary butylic alcohol/distilled water (3:1) was used, the chromatographies were developed in a saturated environment for about 24 h. The various BSP fractions were revealed by ammonia vapours or a mixture of 1 part of 10% NaOH and 9 parts of methanol.

**Results.** The Figure shows the results of chromatographs of the urine of normal rats injected i.v. respectively with 5 and 20 mg/kg of BSP at intervals of 60, 120, 240, 360 min and 24 h. By administering 5 mg/kg of dye, free BSP is found in the 60 min sample and traces in 2 following samples. In the first and second sample, evident traces of a metabolite with  $Rf_{BSP} = 0.60$  appear. By administering 20 mg/kg of BSP one sees a different pattern of excretion and the appearance of more numerous metabolites having different  $Rf_{BSP}$ . In fact in all the samples (as long as the 24th h was included) free BSP was found. In the first 60 min sample, 3 metabolites of  $Rf_{BSP} = 0.71$ , 0.61 and 0.39 appear. The metabolite with  $Rf_{BSP} = 0.71$  appears in traces in the following sample too, while that with  $Rf_{BSP} = 0.61$  is clearly visible in the second sample. The metabolite with  $Rf_{BSP} = 0.39$  appears in the first sample only.

**Conclusions.** By the use of chromatographic plates of double the length of those commonly used, the run time increase resulting from the use of such plates and by the employ of different doses of BSP, characteristic chromatographic patterns of urinary elimination of BSP can be

**Riassunto.** E' stata eseguita un'indagine mediante una nuova tecnica cromatografica sull'eliminazione urinaria della BSF nel ratto normale in condizioni fisiologiche, dopo somministrazione endovenosa di 5 e 20 mg/kg di colorante. Si è dimostrato che con il variare del dosaggio si hanno modi d'eliminazione completamente diversi in termini temporali e qualitativi.

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