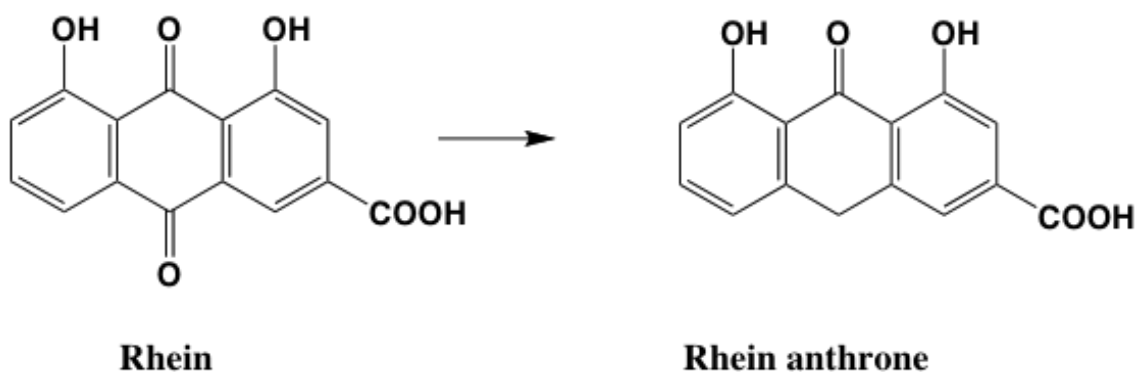


## Rhein



Conversion of rhein by human intestinal bacteria

代謝実験

腸内細菌代謝 ヒト腸内細菌 *Bacteroides* spp. RHEIN-I and RHEIN-II

動物 ラット

### Preparation of an intestinal bacteria mixture and determination of anthrone

A fresh stool sample (1 g) obtained from a healthy man was suspended in 5 ml of 50 mM K-phosphate buffer, pH 7.3. The suspension was filtered with a piece of gauze and made up to 100 ml with the same buffer. This suspension was used as an intestinal bacterial mixture. For quantitative determination of rhein anthrone, *N,N*-dimethyl-*p*-nitrosoaniline (DMPA) in pyridine were added to the culture and mixed. The mixture was then extracted with butanol saturated with H<sub>2</sub>O (200 μl). Five microlitres of the BuOH layer were applied to a TLC plate, which was developed with CHCl<sub>3</sub>-MeOH (7:3). Rhein-metabolizing activity was monitored visually by observing a bluish-green spot (R/ 0.6). [Meselhy *et al.*, *J. Trad. Med.*, **18**, 169-176 (2001)]

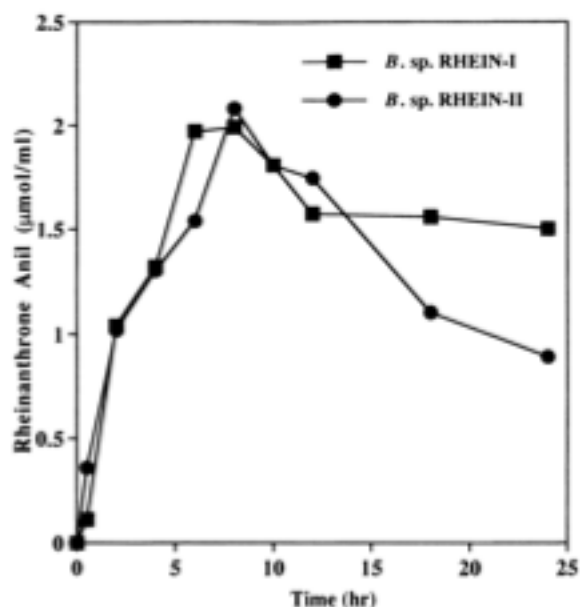


Fig. 1 Transformation of rhein to rheinanthrone by *Bacteroides* spp. strains RHEIN-I and RHEIN-II

Suspensions (500/1) of *B. sp.* RHEIN-I and RHEIN-II were separately added to GAM broth (100 ml) and incubated for 24 hr under anaerobic conditions. Individual cultures were centrifuged at 1500 x g for 15 min and the pellets were washed with 50 mM K-phosphate buffer (pH 7.3, 15 ml), and re-suspended in the same buffer (2 ml). Rhein (in 1% NaHCO<sub>3</sub> solution) was separately added to each bacterial suspension to give 4.9 µmol/ml of the compound, and the mixture was incubated under anaerobic conditions. A portion (200/1) of the culture was taken out at intervals and rhein anthrone was determined as an azomethine derivative by TLC-densitometry at a wavelength of 660 nm relative to a reference wavelength of 780 nm, using a calibration line of an authentic azomethine derivative of rheinanthrone. The calibration line was linear in a range of 2.48-20 nmol/spot. [Meselhy *et al.*, *J. Trad. Med.*, **18**, 169-176 (2001)]

#### 参考文献

- 1) Meselhy M. R., Nishimoto E., Akao T. and Hattori M.: Human intestinal *Bacteroides* spp. RHEIN-I and RHEIN-II capable of transforming rhein to rheinanthrone, induce rhein-dependent diarrhea in rats. *J. Trad. Med.*, **18**, 169-176

(2001).