to amplify a 820 base-pair region of the *ureC* gene. The PCR products were digested with the restriction endonucleases *Sau*3A and *Cfo*1, and the fragments generated were analyzed by agarose gel electrophoresis. Presence of multiple strains of *H pylori* was defined when the sum of the restriction fragments exceeded 820 bp.
Results: *H pylori* could be isolated from 28 patients (20 from gastric biopsy and 8 from gastric aspirate samples); PCR on *H. pylori* genomic DNA was positive in all of them. When PCR was done directly from gastric biopsy/aspirate samples, 24 (18 from gastric biopsy and 6 from gastric aspirate samples) of these patients were positive. No false-positive result was noted. Five RFLP patterns with *Sau*3A and 3 RFLP patterns with *Cfo*1 were identified. RFLP patterns suggesting presence of multiple strains were noted in 3 patients, when PCR was done on genomic DNA from *H pylori* isolates. PCR-RFLP patterns directly from gastric biopsies and aspirates also identified these 3 patients as harboring multiple strains, and was indicative of single strains in the rest 21 patients.
Conclusions: These results indicate that PCR amplifying the 820-bp region of *ureC* directly from gastric biopsy and gastric aspirate samples is highly specific (100%) compared to that from *H pylori* genomic DNA; however the sensitivity is 86%. PCR-RFLP analysis from *H pylori* genomic DNA and directly from gastric biopsy and gastric aspirate samples is equally sensitive in detecting simultaneous gastric colonization by multiple strains of *H pylori.*

● **G0269**

EXTRA VIRGIN OLIVE OIL-ENRICHED DIETS PROTECTS THE NSAID-INDUCED GASTRIC DAMAGE IN RATS: ROLE OF LEUKOCYTE ADHERENCE. Barranco M.D., Alarcón de la Lastra C., Motilva V., Martín M.J., *García-Mauriño S., *Sánchez-Margalet V., *Esteban J., *Herrerías J.M. Dept. de Farmacología, Facultad de Farmacia y Hospital Universitario Virgen Macarena, Universidad de Sevilla, Sevilla, Spain.

BACKGROUND. The Mediterranean diet, which is characterized by a high intake of antioxidants, cereals and olive oil, is reputed to have anti-inflammatory properties. Olive oil contains a small amount n-6 polyunsaturated fatty acid but the highest concentration of oleic acid, a monounsaturated fatty acid, of all edible oils. Polyphenolic compounds are also present in the extra virgin olive oil (unrefined olive oil from olives of good quality) and there is an interest because their antioxidant activities. Previous studies of possible mechanisms of phenol action indicate that these compounds are able to scavenge free radical and to break peroxidative chain reaction. In addition, polyphenols exert several indirect effects reducing the production of chemotactic and inflammatory compounds. Ulceration in the gastrointestinal tract induced by NSAID is the major limitation to their therapeutical use. A vascular etiology has been proposed with activation of polymorphonuclear leukocytes. Neutrophil activation also induces changes in the repertoire of cell surface adhesion receptors and expression of the integrins are involved in neutrophil extravasation during inflammation. Margination of circulating PMN into the gastric microcirculation is an early and critical event in the pathogenesis of NSAID.
AIMS. To examine the hypothesis that diets supplemented with extra virgin olive oil may reduce the severity of the NSAID induced gastric lesion and to explore the effect of some olive oil polyphenols on quantitative and qualitative changes in leukocyte adhesion receptors.
METHODS. Weanling rats were maintained on semisynthetic diets for 6 weeks; standard diet containing 5% (w/w) of fat as control and olive oil suplemmented diets (5% and 20% w/w). Gastric lesion was induced on the last day by oral administration of indomethacin (IND 60 mg/Kg b.w.). The leukocye infiltration in gastric wall was measuring by the myeloperoxidase activity (MPO). The expression of integrins during neutrophil activation with FMLP was assessed by flow cytometry and the following Mab were used: TP1/40 anti-CD11a, Bear-1 anti-CD11b, HC1/1 anti CD11c, and KIM127 anti-CD18. The polyphenolic compounds assayed (25 μM - 1 mM) were: oleuropein (OLR) and caffeic (CAF), syringic (SYR) and protocatechuic (PRT) acids.
RESULTS. In animals consuming standard diet, the total area of lesions was 14.7 ± 3.4 mm^2. In contrast, in animals fed olive oil diets gastric damage decreased in magnitude in parallel with the dietary content in the fat. The ulcer index was decreased to 7.7 ± 1.9 mm^2 ($p < 0.01$ vs IND standard diet) feeding of 5% olive oil enriched diet and to 2.7 ± 0.8 mm^2 in animals consuming 20% olive oil diet ($p < 0.001$ vs IND standard diet). These protective effect were specifically related to a reduction of neutrophil infiltration (MPO values). CAF, SYR and PRT induced a dramatic decrease of CD11b and CD11c expression ($p < 0.001$), whereas a moderate decrease was observed with OLR (CD 11c, $p < 0.05$). In contrast, the expression of other adhesion molecules was unaffected (CD11a, CD18).
CONCLUSION. Results demonstrate the preventive properties of extra virgin olive oil diets in NSAID induced gastric mucosal injury. This effect could be explained by its *in vivo* antinflammatory properties but also by the reduction of the *in vitro* expression of cell adhesion molecules.

G0270

***H. PYLORI* INFECTED MUCOSA IN GASTRIC ULCER SAMPLES: PHOSPHOLIPASE ALCOHOL DEHYDROGENASE AND UREASE ACTIVITIES.** R. Barreto-Zuñiga[1,3], M. Okuyama[2], Y. Kato[3], F. Marotta[3,6], H. Ohta[4], T. Takekoshi[3], M. Maruyama[3], D. Murguia[1]. GI Service Mexico General Hosp.[1], Instit. of Whole Body Metabolism, Chiba, Japan[2], GI Service, S. Anna Hosp., Como, Italy.[6], Int. Med.[3], Surgery[4] and Pathology[5] Dept. Cancer Institute Hosp. Tokyo, Japan.

Obective: At mucosal surface *H. pylori* (HP) enzymes generate toxic molecules: ammonia (urease, UR), lysolecithin (phospholipase, PL) and acetaldehyde (alcohol dehydrogenase, ADH). We investigated whether UR, PL and ADH activities are alterated in the gastric mucosa from gastric ulcer (GU), compared with controls. **Methods:** Biopsy taken from 44 GU and 73 controls, comprising two subgroups: 48 non ulcer patients (NUG) and 24 partial gastrectomy patients (PG) as gastric injured control. The HP status were detected by culture, *in vitro* urease and histological tests. Enzyme activities were detected by newly radiotracer technique TLC-Autoradioluminography (TLC-ARLG) **Results:** The mean of enzymes levels in HP positive samples shows statistical significantly differences, than HP negative. [*Pi: Student's t test for paired data; Prob. (2-tail)].

	H. pylori (+)	*H. pylori* (-)	
	Mean ± SE	Mean ± SE	Pi
PL	.116 ± .22	.179 ± .38	.09*
ADH	.138 ± .106	.195 ± .12	.01*
Urease	5.79 ± 3.73	.598 ± .676	.004*

The most evident alterations of PL were induced by PG and UG (84% and 34% less than NUG respectively). The GU samples infected with HP had significantly lower ADH (mean GU=0.151 vs. NGU=0.285 pCi/mg/min) and PL activities [(mean GU=0.116 vs. NGU=0.179 (min-1X100)] than NGU samples. The mean urease levels in HP positive samples were significantly higher than HP negative samples (Table). Alteration of enzyme activities were well correlated with the degree of mucosal changes such as mononuclear or polymorphonuclear cell infiltration. **Conclusion:** The UR, PL and ADH enzymatic profiles, reflect the pathological adaptations behind mucosal injury in UP and PG. Although high activity of UR indicates well the presence of HP, impairment activities of PL and ADH reflect more the gastric mucosal inflammation than HP infection "per se". Further studies should be primarily attempted with TLC- ARLG in patients with gastric disease and HP treatment.
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● **G0271**

INDOMETHACIN (Indo) AND BILE SALTS (BS) COMPETE FOR THE BILIARY PHOSPHATIDYCHOLINE (PC): AN EXPLANATION OF Indo-INDUCED INTESTINAL INJURY. JM Barrios[o] and LM Lichtenberger*, [o]Department of Pediatrics, Baylor College, Houston TX and *Department of Integrative Biology, University of Texas Medical School, Houston TX.

Background: PC represents ± 40% of the organic material of bile, PC has the capacity of associating with non-steroidal anti-inflammatory drugs (NSAIDs) and bile salts, decreasing the GI toxicity of these two classes of compounds. NSAIDs that undergo enterohepatic cycling are toxic to the ileal mucosa, by a mechanism yet to be elucidated.
Hypothesis: Biliary PC associates with and detoxifies bile salts, forming mixed micelles. NSAIDs secreted into the bile compete with bile salts for the available PC, resulting in increase in the concentration of free bile salt to damage the intestinal mucosa.
Methods: 5mM of Deoxycholic acid, Indo and PC, were instilled into a loop of the distal ileum of anesthetized rats, alone and in combination. After 30 minutes, loop fluid and ileal mucosa were collected for hemoglobin (Hb) and contact angle analysis. The same combinations were used to assess their effect on human erythrocytes (RBCs) as measured by degree of hemolysis, except PC was administered at both 5 and 10mM.
Results: are shown below with *=$p < 0.05$ vs saline/buffer.

	Saline	BS	PC	
Ileal Hydrophobicity (Contact θ)				
Saline	26.8 ± 1.6	8.7 ± 1.3*	30.5 ± 2.1	
Indo	23.5 ± 3.2	20.3 ± 3.4	14.1 ± 2.6*	
Ileal Loop Hemoglobin Concentration (mg %)				
Saline	5.5 ± 1.6	52.7 ± 18.8*	5.7 ± 1.0	
Indo	3.0 ± 1.0	9.8 ± 3.8	17.8 ± 5.0*	

RBC Hemolysis				
	Saline	BS	BS + 5PC	BS + 10PC
Saline	0	8.4 ± 1.5*	0.7 ± 0.1	0.7 ± 0.3
Indo	0	8.8 ± 1.3*	4.8 ± 2.6*	0.5 ± 0.6

In both, *in vivo* and in *in vitro* experiments the protective effects of PC against bile salt-induced injury were reversed by Indo.
Conclusion: These findings confirm our hypothesis that PC protects against the injurious action of bile salts on cell membranes. Indo and perhaps other NSAIDs that enter bile, damage the mucosa, not by a direct toxic action, but by competing for the available protective PC molecules.