1H), 3.85 (d, J = 8.75 Hz 2H), 3.65 (m, 2H), 2.55 (q, J = 10 Hz, 1H), 1.88 (s, 3H), 1.75 (d, J = 2.5 Hz, 3H), 0.88 (m, 3H), 3.95, 3.58, 2.05 (1H each exchangeable with D_2O ppm). These data were consistent with compound **1** being an aromatic diester of 5,12-dihydroxy-6,7-epoxy-resiniferonol similar to mezerein[3] from *Daphne mazereum* where 1 acyl group was present in the O-acyl form whilst the other was present in the ortho-ester configuration[2].

Fraction 2 from the column was initially purified by TLC on silica gel using chloroform/ethyl acetate (20/30) as solvent (R_f 0.45), and finally by partition TLC as before using cyclohexane/butanone (80/20) as solvent (R_f 0.30).

Compound **2** was obtained as a glassy resin in a yield of 30 mg; the IR spectrum was similar to that of compound **1**; EI-MS (190 °C, 40 e.v.), m/z 748 ($M^{+\cdot}$, 1%), 481 (30%). 358 (15%), 340 (20%), 322 (25%), 317 (22%), 281 (60%), 105 (100%); ^{1}H-NMR ($CDCl_3$, 80 MHz, TMS = 0.00 ppm), δ 7.82–7.10 (5H), 7.58 (bs, 1H), 6.50–5.71 (2H), 5.18 (s, 1H), 5.05 (s, 2H), 4.95 (d, J = 3.5 Hz, 1H), 4.25 (s, 1H), 3.90 (m, 1H), 3.85 (d, J = 8.8 Hz, 2H), 3.65 (m, 2H), 2.55 (q, J = 10 Hz, 1H), 2.10 (m, 2H), 1.88 (s, 3H), 1.75 (m, 3H), 1.20 (bs, 26H), 0.99 (t, 3H), 0.88 (m, 3H) ppm. Compound **2** accordingly was related to **1** but differed from it in that one of the acyl substituents was aliphatic in nature. This derivative therefore belonged to a group of ortho-esters which include Gnididin and Gniditrin previously isolated from *Gnidia lamprantha*[2,9].

The question remained as to which of the acyl substituents in both compound **1** and **2** were in the O-acyl configuration and which were present as the ortho-esters. Compounds **1** and **2** were separately hydrolyzed with 1% sodium methoxide in methanol to produce the common reaction product **3**; EI-MS (170 °C, 40 e.v.), m/z 498 ($M^{+\cdot}$, 1%, $C_{27}H_{30}O_9$); ^{1}H-NMR ($CDCl_3$, 80 MH_z, TMS = 0.00 ppm), δ 7.80–7.30 (5H), 7.56 (bs, 1H), 5.13 (s, 2H), 4.85 (d, J = 3.5 Hz, 1H), 4.25 (s, 1H), 4.20 (s, 1H), 3.90 (s, 1H), 3.85 (d, J = 8.8 Hz, 2H), 3.65 (m, 2H), 2.55 (q, J = 10 Hz, 1H), 1.88 (s, 3H), 1.75 (m, 3H), 0.88 (m, 3H) ppm. This reaction product was 5,12-dihydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate[3]. A previous X-ray diffraction study of molecular structure and conformation of an O-acyl derivative of this product confirmed its constitution as an ortho-ester[10]. When **3** was acetylated with acetic anhydride/pyridine (2/1) an acetate **4** was produced which from the characteristic shifts of protons on C-5, 12 and 20 together with the appearance of three 3H signals at 2.0-2.1 ppm in this ^{1}H-NMR spectrum confirmed **4** as the 5, 12, 20-triacetate of **3**[2]. On the basis of these data **1** was assigned as 12-O-cinnomoyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate and **2** was 12-O-heptadecenoyl-5-hydroxy-6,7-epoxy-resiniferonol-9,13,14-orthobenzoate. Compounds **1** and **2** were the major toxic diterpenes from Egyptian *Thymelaea hirsuta* L. These compounds induced erythema in 100% of test animals in a dose of 0.1 µg when tested for pro-inflammatory activity on 2 groups of 36 animals using the mouse-ear model[5].

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Inhibitory effect of ouabain on in vitro and in vivo gastric acid secretion in the frog and the rat

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Summary. The in vitro and in vivo effects of ouabain on gastric acid secretion in the frog and the rat, the 2 species known to have different sensitivity to ouabain, were studied. It was found that ouabain was a potent inhibitor of histamine-stimulated acid secretion in the isolated frog gastric mucosa. Ouabain administered i.v. at dose levels far below the lethal range also produced a marked and significant reduction of histamine-stimulated gastric acid secretion in the anesthetized frogs and rats. It is considered that the inhibitory effect of ouabain on acid secretion could be partly related to its specific antagonizing action on the Na^+-K^+-ATPase in the gastric mucosa.

Ouabain, one of the cardiac glycosides, is a well-known specific inhibitor of the sodium- and potassium- activated adenosine triphosphatase (Na^+-K^+-ATPase). Despite a considerable amount of work carried out to study the mode of action of ouabain on the gastric mucosa, published results concerning the effect of ouabain on gastric acid secretion remain controversal[2-5]. The aim of the present study was to investigate more fully the in vitro and in vivo effect of ouabain on histamine-stimulated gastric acid secretion in the frog. In addition, comparative studies were performed to evaluate the action of ouabain on histamine-stimulated gastric acid secretion in the rat, a species found to be relatively insensitive to ouabain[6].

Materials and methods. Rana temporaria temporaria L., b.wt about 30 g, was used in all experiments on frog gastric acid secretion. For in vitro experiments, the frog gastric mucosa free of muscle layers was mounted between 2 glass chambers. 50 ml of serosal and mucosal solution were used to bathe the serosa and mucosa respectively. The serosal solution contained (mM): NaCl 85.3, KCl 3.4, KH_2PO_4 0.9, $MgSO_4 \cdot 7H_2O$ 0.9, $CaCl_2$ 1.8, $NaHCO_3$ 1.76 and glucose 0.2%. The mucosal solution had the same composition as the serosal solution except that $NaHCO_3$ was replaced by equimolar NaCl. Both solutions were gassed with 100% O_2. The mucosal solution was changed every 30 min. The experiments were performed at room temperature (20–22 °C). Drugs were added to the serosa and expressed as the final concentration in the serosal solution. For whole animal experiments, the frogs were anesthetized by half-immersion in a solution containing 0.1% MS 222 Sandoz

(ethyl meta-amino benzoate) for at least 45 min; the animals were then weighed and kept anesthetized throughout the experiment by placing cotton-wool soaked with MS 222 solution in contact with the frog's skin. Both the cardiac and pyloric ends of the frog stomach were cannulated with polythene tubing and the stomach was perfused with 0.7% NaCl solution at room temperature. The Ghosh and Schild method[7] was followed to perform in vivo experiments in rats. Male Wistar rats, b.wt between 180–200 g, were anesthetized with urethane and the stomach was perfused with 0.9% NaCl solution at 37°C. For both the frog and rat stomach, the perfusion rate was 0.17 ml/min., and the gastric perfusate was collected at 30-min intervals. Histamine was either instilled into the intact frog stomach or given s.c. to the anesthetized rat. Ouabain was administered by slow i.v. injection into the abdominal vein of the frog or the femoral vein of the rat. In each experiment, basal secretion was followed for 2 h and thereafter the effect of drug was observed for another 4–5 h. All gastric samples were titrated to pH 7.0 with 10^{-2} M NaOH by means of a Radiometer Autotitrator. The results are expressed as acid output (μmole/30 min or h.). Student's t-test was used to evaluate the results and a p-value of less than 0.05 was considered significant. All chemicals used were of the A.R. grade. Histamine acid phosphate and ouabain (G-strophanthin) were obtained from B.D.H. Ltd. The dose of histamine was expressed in terms of its base. Ouabain and histamine were dissolved in the serosal solution or saline and administered in a volume of not more than 0.3 ml.

Results and discussion. Three groups each consisting of 6 isolated frog gastric mucosae were used to study the effect of ouabain on histamine-stimulated acid secretion. The mean rate of acid secretion (acid output, μmole/h, mean and SE) of all preparations before treatment was 0.24 ± 0.04 and this rose to 0.36 ± 0.12 and 1.11 ± 0.09 in the mucosae treated with saline and histamine (2.5×10^{-4} M), respectively. It can be seen from figure 1 that in gastric mucosae receiving ouabain (10^{-3} M) 30 min prior to histamine (2.5×10^{-4} M), acid output was reduced to 0.15 ± 0.08 μmole/h, thus showing that ouabain produced significant inhibition ($p < 0.001$) of histamine-stimulated acid secretion in the isolated frog gastric mucosa.

A series of preliminary experiments were carried out to determine the suitable dose level and route of administration of histamine and ouabain for the in vivo study. Figure 2 shows the results of 6 experiments each on the effect of histamine on gastric acid secretion in the anesthetized frogs in the absence and presence of ouabain. It is clear from these results that ouabain at the dose of 100 μg/kg (single slow i.v. injection) significantly decreased the marked and substained stimulatory effect of histamine (1.5 mg/frog, in 0.3 ml 0.7% saline, intragastric instillation).

The effect of ouabain on histamine-stimulated gastric acid secretion in the anesthetized rats was also investigated and the results are plotted in figure 3 as a function of time. Acid secretion (acid output, μmole/30 min, mean and SE) in the 6 rats treated with histamine (1.8 mg/kg, single s.c. injection) increased from the basal rate of 3.0 ± 0.36 to the peak output of 42.18 ± 4.02 whereas the corresponding peak value in other 6 rats receiving ouabain 1 h prior to a similar s.c. dose of histamine was 8.88 ± 1.31. The differences in the value of acid output between the histamine-treated and ouabain + histamine-treated rats were significant throughout the substained peak acid secretory period lasting for 2½ h ($p < 0.01$ to 0.001). In the present experiments with frogs given ouabain at 100 μg/kg i.v. or in rats treated with ouabain at 50 μg/kg as a bolus slow i.v. injection, followed by repeated single slow i.v. injection at 25 μg/kg every 30 min throughout the experimental period, the animals survived and no apparent toxic effects of ouabain on the animals were observed. In the anesthetized rats pre-treated with ouabain, stimulation of acid secretion by histamine was evident. In the anesthetized frogs receiving ouabain and histamine, acid output increased slightly and then decreased to a level lower than that during basal secretion, thus indicating that spontaneous acid secretion was also affected. The results

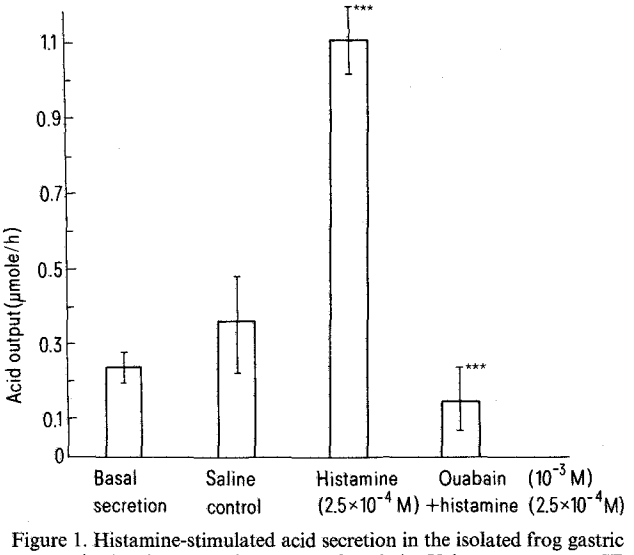

Figure 1. Histamine-stimulated acid secretion in the isolated frog gastric mucosa in the absence and presence of ouabain. Values are mean ± SE of 6 experiments. ***$p < 0.001$, histamine-treated mucosae vs saline control; ***$p < 0.001$, ouabain + histamine-treated mucosae vs mucosae treated with histamine alone. Ouabain administered 30 min before histamine addition.

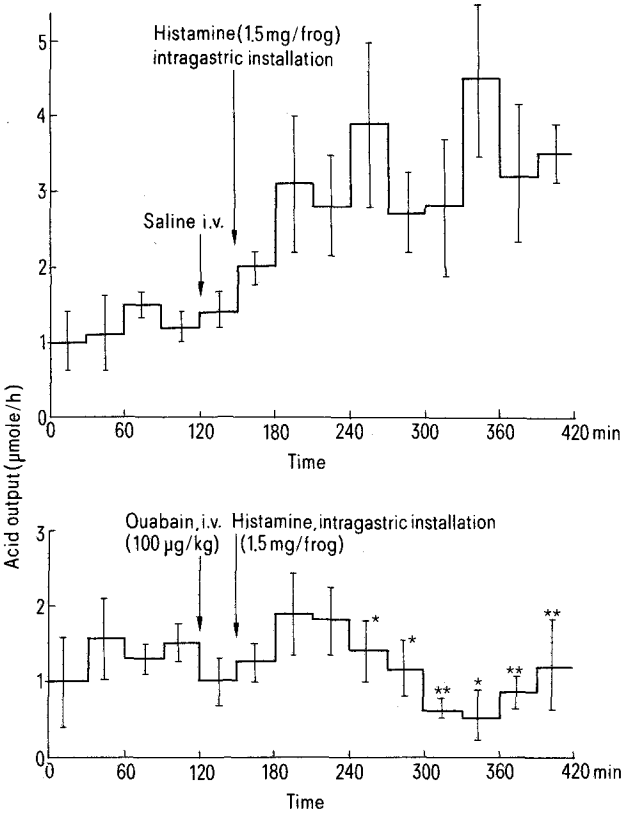

Figure 2. The effect of ouabain on histamine-stimulated gastric acid secretion in anesthetized frogs. Values are mean ± SE of 6 experiments. *$p < 0.05$, **$p < 0.01$, acid output from ouabain + histamine-treated frogs vs that from frogs receiving saline + histamine.

shown in figure 1 also suggest that spontaneous acid secretion in vitro was inhibited by ouabain, and this finding is in agreement with the results reported by Davenport[2].

The present study provides evidence that ouabain is a potent inhibitor of histamine-stimulated acid secretion in the isolated frog gastric mucosa. The inhibitory action of ouabain on acid secretion in the isolated gastric mucosa of the rat[3] and the dog[5] has been reported. It is of particular interest to note that in the isolated rabbit gastric glands, ouabain also produced marked inhibition of cAMP- and histamine-stimulated acid secretion monitored as a function of the accumulation of the weak base (^{14}C aminopyrine)[8]. There are very few published reports of the in vivo effects of ouabain on gastric acid secretion. Kuo et al[9] found that i.v. injection of ouabain at 50 µg/kg (approximately i.v. LD_{50} for dogs) did not alter acid secretion in the canine gastric flap preparation in vivo. The LD_{100} i.v. as µg/kg for the rat and for the frog was shown to be 77, 110 and 600, respectively[10]. In the present study, an i.v. administration of ouabain at 100 µg/kg for the frog and at 50 µg/kg for the rat, a dose level far below the lethal range, was shown to produce marked and significant reduction of gastric acid secretion in these 2 species. The discrepancy in the results obtained could be due to the different sensitivity of various species and organs to the action of ouabain[6].

Ouabain has been used as a specific marker of the Na^+ -K^+ -ATPase in a variety of biological tissues and cells[11-13]. It is generally agreed that, in the isolated gastric mucosa, ouabain inhibition of the gastric mucosal Na^+-K^+-ATPase caused an increase and a decrease of intracellular Na^+ and K^+, respectively; which in turn led to the reduction of acid secretion. K^+ is essential and Na^+ is inhibitory for the acid secretory process and the role of the Na^+ -K^+ -ATPase is to maintain a normal level of intracellular K^+ (or the Na^+/K^+ ratios)[13-16]. It has been shown that the Na^+ -K^+-ATPase is located at the basal and lateral surfaces of the oxyntic cells and has characteristics quite distinct from the ouabain uninhibitable K^+-activated ATPase found in the gastric mucosa of various species (frog, dog, hog, rabbit and man)[13,17]. Based on results of experiments with isolated vesicles and glands from gastric mucosa, several investigators and their co-workers have presented a H^+/K^+ exchange model to explain the mechanism(s) of gastric acid secretion[15-17]. Briefly, it is considered that the K^+-activated ATPase is a major component of the ATP-dependent H^+/K^+ pump which plays a central role in hydrogen ion secretion. The K^+-activated ATPase is located at the apical surface of the oxyntic cells and K^+ originating from cytoplasm activates the K^+-activated ATPase by binding to a K^+-selective site on the luminal surface of the pump; K^+ is then recycled back into the cytoplasm for the exchange of H^+ derived presumably from water. It is also postulated that Cl^- is actively transported by a Na^+-dependent step at the serosal membrane into the cytoplasm of the oxyntic cells and then moves passively down an electrochemical gradient across the mucosal surface for the formation of HCl with H^+ that has been actively secreted into the gastric lumen via the work of the H^+/K^+ pump.

It seems clear from the present study that the inhibitory effect of ouabain on vitro acid secretion could be partly related to its specific antagonizing action on the Na^+ -K^+ -ATPase in the gastric mucosa. It is uncertain, however, whether this same inhibitory mechanism is attributable to ouabain antagonism of in vivo gastric acid secretion. It has been known for a long time that cardiac glycosides affect the sympathetic and parasympathetic nervous system and cause the release of secretory products, neurotransmitters and hormones from most tissues[18,19]. Ouabain has been found to release catecholamines from various tissues including the adrenal gland and the gut nerves[19,20]. Isoprenaline (a β-adrenoceptor agonist) and phenylephrine (a α-adrenoceptor agonist) are potent inhibitors of gastric acid secretion in the intact rat and dog[21,22], and it is possible that ouabain induced its inhibitory action on gastric acid secretion in the anesthetized frogs and rats by releasing catecholamines from the stomach and extragastric origin. Ouabain has effects on other cellular functions such as the uptake of amino acids, the distribution of sodium and calcium ions, and protein synthesis[23-25], and it is important to bear this in mind when interpretations are made on the mode of action of ouabain on the gastric mucosa.

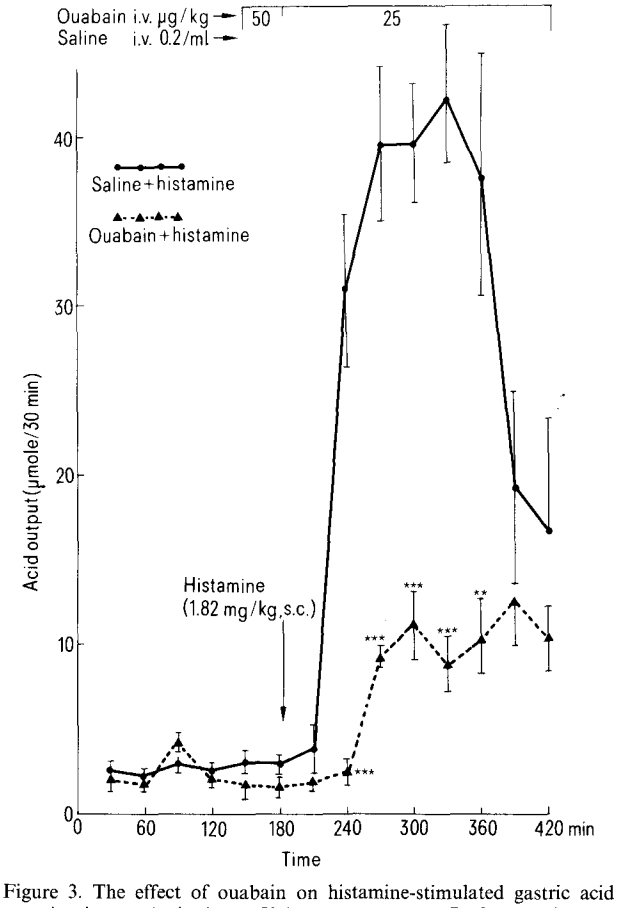

Figure 3. The effect of ouabain on histamine-stimulated gastric acid secretion in anesthetized rats. Values are mean ± SE of 6 experiments. **$p < 0.01$, ***$p < 0.001$, acid output from ouabain + histamine-treated rats vs that from rats receiving saline + histamine.

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K-p-Nitrophenylphosphatase activity, Na and K content, Na permeability and membrane lipid composition in rabbit myocardium after cholesterol rich diet

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Summary. The aim of the present study was to investigate the effects of a cholesterol-rich diet on membrane function and lipid composition in rabbit myocardium. The activity and the ouabain sensitivity of the K-p-nitrophenylphosphatase (K-pNPPase), a partial reaction of the Na,K-ATPase, were diminished after a cholesterol/oil or pure cholesterol diet. The content of cholesterol, cholesterol esters and of several classes of phospholipids was enhanced in microsomes. A causal relationship is assumed between cholesterol accumulation and a decrease in membrane fluidity as well as in Na,K-ATPase activity. The intracellular Na content and the Na-Li-exchange rate were higher after the cholesterol diet. The increase in the Na content is supposed to be induced by a lower Na transport and a higher Na permeability. An enhanced Ca flux via the sarcolemma could be the consequence.

The biochemical and physiological functions as well as the physicochemical properties of membranes strongly depend on their lipid and cholesterol content. Variations in the cholesterol content or in the phospholipid composition of the lipid bilayer, caused e.g. by genetic factors, nutritional changes and the aging process, lead to alterations in membrane fluidity and lipid-protein interactions[2,3]. Thereby the permeability of ion channels and the activity of enzymes could also be influenced[4-7]. In this connection the contraction and relaxation of the isolated rabbit papillary muscle are influenced as a consequence of an altered Ca regulation in the heart after a cholesterol rich diet[8].

Na and Ca fluxes through the sarcolemma are functionally coupled[9]. Intracellular Na enrichment is also accompanied by Ca accumulation favoring the development of myocardial necrosis[10]. Therefore we investigated whether a cholesterol rich diet can modify mechanisms which are involved in cellular Na regulation. The K-p-nitrophenylphosphatase activity (K-pNPPase), the Na and K content and the Na-Li-exchange rate of the myocardium were measured after the diet. In microsomes and mitochondria the content of phospholipids and cholesterol was investigated.

Materials and methods. Hearts were obtained from rabbits fed for 12 weeks with a diet of standard pellets supplemented with either 2 g cholesterol or 2 g cholesterol mixed with 20 ml oil. In each test series data obtained from hearts of rabbits fed with standard pellets were used as control. After sacrifice by a blow on the head the heart was quickly removed, rinsed and freed of connective tissue and fat. About 2 g of the ventricular myocardium were frozen rapidly in liquid nitrogen and kept at −20 °C. The homogenization and the determination of the K-pNPPase activity were performed as described by Lamers et al.[11]. On the day of homogenization, the ventricle was weighed, dissected into small pieces and homogenized with 5 volumes of 0.25 M sucrose, 5 mM EGTA, 5 mM Tris-HCl (pH 7.0) in a Virtis 45 homogenizer at half-maximal speed. Homogenization was carried out for 4 periods of 5 sec alternating with 120 sec of cooling. After this, the homogenate was passed through a nylon filter under light pressure. All procedures were done on ice.

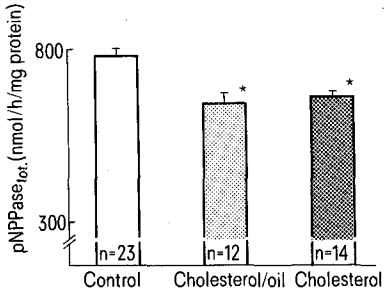

Figure 1. The total K-p-Nitrophenylphosphatase activity (pNPPase$_{tot}$) after the cholesterol and the cholesterol/oil diets; 2 $p \leq 0.05$.

Table 1. The Na and K content and the Na-Li-exchange rate (D) of the myocardium after cholesterol-rich diets

	Cholesterol/ diet (n = 10)	Control (n = 12)	Cholesterol/ oil diet (n = 15)	Control (n = 13)
Na (mmol/kg wet wt)	43.8 ± 4.5	33.5 ± 3.3	35.4 ± 0.1*	32.0 ± 0.4
K (mmol/kg wet wt)	66.7 ± 3.3	68.7 ± 2.8	61.8 ± 1.6	65.0 ± 2.2
K/Na	1.4 ± 0.05*	2.0 ± 0.10	1.8 ± 0.06*	2.1 ± 0.05
D	0.09 ± 0.02*	0.01 ± 0.02	–	–

* $2p \leq 0.05$.