insure complete administration, blood was withdrawn into the syringe before and after injection of BSP. Any animal that appeared to be traumatized by the procedure was not used. Pentobarbital sodium (50 mg/kg, i.p.) was given 25 min after the BSP. 5 min later, the abdominal cavity was opened and blood collected from the heart. Then, the liver was removed, blotted with a gauze pad and weighed. Serum levels of BSP were determined by the method of Seligson[17]. To calculate the 100% BSP retention, the plasma volume was assumed to be 5.7% of the body weight[18]. BSP storage was estimated by the method of Whelan et al.[19] and the level of serum aspartate aminotransferase (GOT) was assayed as described previously[20].

Results and discussion. At the dose used in this study, the endotoxin (LPS) killed 4 of 8 treated 1-day-old guinea-pigs within 48 h, but did not cause any mortality in the 3-day-group (5 animals). There were no differences in % BSP retention, at 30 min, among the control groups at 1, 3 and 7 days. The storage of BSP in the liver decreased from 1 to 7 days, indicating maturation of hepatic conjugative processes[15]. At 1 day, BSP in the liver accounted for about 21% of the applied dose, but at 3 and 7 days, only about 4% (table). These data are in agreement with those reported by other authors[15,16].

Administration of the LPS increased the percent BSP retention in the 1- and 3-day-groups, but not the 7-day-group. At all ages studied, the LPS caused an increased storage of BSP in the liver, whether expressed in μg/g liver or as percent of the applied dose (table). The data suggested that the LPS interfered with hepatic function as the excretory level as there were increased levels of BSP in the serum and liver in the 1- and 3-day-old guinea-pigs. Although it is possible the LPS reduced dye excretion by inhibiting BSP conjugation, the observation that LPS inhibited the excretion of indocyanine green[8], indicates this is unlikely. The results of the present study are in agreement with those reported for the IPRL[8,9], that the LPS exerts adverse effects on hepatic excretory mechanisms. There was a moderate rise in serum GOT in the 1-day-group, but not in the 3- and 7-day-groups (table). The above results indicated that newborn guinea-pigs are more sensitive to LPS than are older animals.

These results may possibly have relevance to the clinical syndrome of intrahepatic cholestasis associated with non-hepatic gram-negative bacterial infections. Since this entity is more prevalent in neonates[3–5] than adults[6], the newborn human, as the guinea-pig, may be more sensitive to LPS than adults. The data of this study are consistent with the hypothesis[8] that the impairment of hepatic excretory processes is a factor in the development of cholestasis often seen during gram-negative bacterial infections.

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Interaction between aldosterone and renomedullary prostaglandins. Competitive action between aspirin and spironolactone

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Summary. Aldosterone injected i.m. decreased the release of renomedullary PGEs and the index (urinary Na/K ratio) in conscious normotensive intact and adrenalectomized rats. Coadministration of spironolactone increased the release of PGEs as well as the index (urinary Na/K ratio). The effect of spironolactone was partly inhibited by aspirin injected in a ratio 5:1 (aspirin:spironolactone), an effect which could be reversed by the infusion of a synthetic prostaglandin (PGA_2) in a subhypotensive dose.

In a previous study in the man[2], we found a positive, statistically significant correlation between urinary PGEs and the index (urinary Na/K ratio) ($Y = 0.015\,X + 0.53$; $r = 0.672$, $p < 0.001$) and it had been suggested that the phenomenon was an antagonistic result between these substances (PGEs) and the aldosterone system. These results enable us to investigate whether the administration of aldosterone in experimental animals could decrease the renomedullary PGEs synthesis and/or release and the coadministration of an antagonist of aldosterone, the spironolactone[3], could increase the release of the natriuretic PGEs. The simultaneous administration of aspirin, a well-known inhibitor of PG synthesis and/or release[4], could further clarify whether the natriuretic effect of spironolactone was only the antagonistic result between these substances and aldosterone or whether it could be mediated (at least in part) by the potent natriuretic renomedullary PGEs[5].

Our results are suggestive of an inhibitory effect of aldosterone on renomedullary PGEs synthesis and/or release and of a stimulating effect of spironolactone on PGEs synthesis.

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Table 1. The effect of aldosterone (1 μg 100 g^{-1} 24 h^{-1}), spironolactone (2 mg 100 g^{-1} 24 h^{-1}), aspirin (10 mg 100 g^{-1} 24 h^{-1}) and prostaglandin A_2(PGA_2) (200 ng kg^{-1} min^{-1}) on urine flow (V), sodium and potassium excretion ($U_{Na}V$ and U_KV) and on the index (urinary Na/K ratio) in the group of the adrenalectomized rats

	n	Control Mean (± SEM)	p	Aldosterone Mean (± SEM)	p	Aldosterone + spironolactone Mean (± SEM)	p	Aldosterone + spironolactone + aspirin Mean (± SEM)	p	Aldosterone + spironolactone + aspirin + PGA_2 Mean (± SEM)
V (ml kg^{-1} h^{-1})	15	1.625 ± 0.338	< 0.15	1.145 ± 0.309	< 0.25	1.430 ± 0.265	< 0.4	1.530 ± 0.196	< 0.4	1.650 ± 0.350
$U_{Na}V$ (μEq kg^{-1} h^{-1})	15	279 ± 51	< 0.05	177 ± 7	< 0.01	312 ± 48	< 0.2	238 ± 58	< 0.15	339 ± 60
U_KV (μEq kg^{-1} h^{-1})	15	97 ± 10	< 0.05	117 ± 7	< 0.01	82 ± 12	< 0.02	117 ± 9	< 0.47	115 ± 25
Na/K	15	2.76 ± 0.42	< 0.01	1.54 ± 0.13	< 0.001	3.85 ± 0.42	< 0.001	1.91 ± 0.39	< 0.001	3.18 ± 0.27
log (Na×10/K)	15	1.421 ± 0.077	< 0.01	1.182 ± 0.039	< 0.001	1.577 ± 0.048	< 0.001	1.208 ± 0.098	< 0.001	1.500 ± 0.078

Material and methods. 2 groups of male Wistar rats, weighing 350–450 g, were studied. The first group (adrenalectomized rats) included 25 animals. The second group (intact rats) consisted of 200 animals. The rats of each group were randomly allocated to 5 and 4 subgroups respectively, each comprising 5 animals, in the group of the adrenalectomized rats, and 50 animals, in the group of the intact rats.

Each subgroup (of both groups) was treated as follows (tables 1 and 2). First subgroups: The animals were untreated (controls). Second subgroups: The rats injected i.m. with D-aldosterone (1 μg 100 g^{-1} 24 h^{-1}) diluted in 0.1 ml of sesame oil. Third subgroups: The animals were treated as those of the second subgroups, in addition they were injected with spironolactone (2 mg 100 g^{-1} 24 h^{-1}).

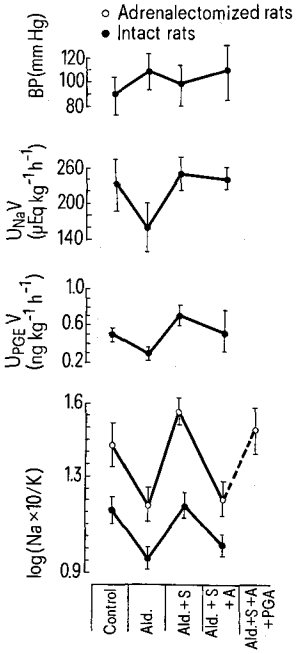

Fig. 1. Effect of aldosterone (Ald), spironolactone (S), aspirin (A) and of the synthetic PGA_2 on the blood pressure (BP), sodium excretion ($U_{Na}V$), renomedullary prostaglandin E release ($U_{PGE}V$) and on the index of (urinary Na/K ratio) expressed as log (Na×10/K) in the rats.

Fourth subgroups: The rats were treated as those of the third subgroups, in addition they were injected with aspirin (in a ratio of 5:1 [aspirin:spironolactone]). Finally, the fifth subgroup, of the first group, was treated as the fourth subgroups, and in addition the animals were infused i.m. with a synthetic prostaglandin (PGA_2) at the subhypotensive dose of 200 ng kg^{-1} min^{-1} (25 μl kg^{-1} min^{-1}) by a Braun continuous infusion pump. All animals were fed standard laboratory chow with free access to water containing 9‰ sodium chloride (saline).

The following parameters were measured (tables 1 and 2).

1. In each subgroup of both groups. Urinary flow (V). Urine was collected by using metabolic cages. Sodium and potassium ($U_{Na}V$ and U_KV μEq kg^{-1} h^{-1}) output were determined by the usual methods. Electrolyte concentrations were measured by a flame photometer.

2. In each subgroup of the second group only. Urinary creatinine and urinary PGEs and PGFs (ng kg^{-1} h^{-1}) were measured by methods described in detail elsewhere[6–8]. The arterial blood pressure was calculated, 3 times in 24 h, as the mean value of 3 subsequent measurements with a Narco Biosystem Electrosphygmomanometer (Huston, Texas, USA) in a period of 30 min. Each subgroup was studied for 5 days, and sodium, potassium and creatinine were determined 3 times in 24 h in aliquots of 4 h (n = 15). Substances having the chromatographic and the bioassay properties of PGEs and PGFs were extracted from 500 ml of 24-h urine collections. The procedure used for extraction, purification, chromatographic separation and quantitative bioassay estimation of PGs is described in detail elsewhere[7–11].

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Results. The results are summarized in tables 1 and 2 and in figure 1. The administration of aldosterone provokes a decrease in sodium and an increase in potassium excretion resulting to a statistically significant increase of the index (urinary Na/K ratio) ($p < 0.01$ and 0.02 respectively, second subgroups tables 1 and 2, figure 1). This result was accompanied by a statistically significant decrease in urinary PGEs concentration ($p < 0.02$, table 2). Arterial blood pressure increased, but not significantly (table 2 and figure 1).

The coadministration of spironolactone provokes an increase in sodium and a decrease in potassium excretion, in both subgroups, resulting in a significant increase of the index (urinary Na/K ratio). A statistically significant increase in urinary PGEs concentration was also observed (tables 1 and 2, figure 1).

When aspirin was added in the injected solution of spironolactone, urinary sodium excretion decreased, urinary potassium excretion increased and the index (urinary Na/K ratio) significantly decreased ($p < 0.001$

Table 2. The effect of aldosterone (1 μg 100 g^{-1} 24 h^{-1}), spironolactone (2 mg 100 g^{-1} 24 h^{-1}) and aspirin (10 mg 100 g^{-1} 24 h^{-1}) on urine flow (V), blood pressure (BP), sodium and potassium excretion ($U_{Na}V$ and U_KV), renomedullary prostaglandin release ($U_{PGE}V$ and $U_{PGF}V$) and on the index (urinary Na/K ratio) in the group of normal rats

	n	Control Mean (± SEM)	p	Aldosterone Mean (± SEM)	p	Aldosterone + spironolactone Mean (± SEM)	p	Aldosterone + spironolactone + aspirin Mean (± SEM)
V (ml kg^{-1} h^{-1})	15	1.657 ± 0.218	< 0.1	1.248 ± 0.171	< 0.05	1.982 ± 0.336	< 0.15	2.454 ± 0.250
Creatinine (mg kg^{-1} h^{-1})	15	1.550 ± 0.223	< 0.4	1.642 ± 0.298	< 0.45	1.688 ± 0.224	< 0.45	1.671 ± 0.250
$U_{Na}V$ (μEq kg^{-1} h^{-1})	15	233 ± 44	< 0.1	156 ± 40	< 0.05	252 ± 31	< 0.45	244 ± 17
U_KV (μEq kg^{-1} h^{-1})	15	175 ± 41	< 0.45	185 ± 12	< 0.2	161 ± 26	< 0.02	259 ± 34
Na / K	15	1.430 ± 0.200	< 0.02	0.895 ± 0.077	< 0.001	1.502 ± 0.132	< 0.01	1.038 ± 0.114
log (Na × 10/K)	15	1.144 ± 0.059	< 0.02	0.946 ± 0.034	< 0.001	1.170 ± 0.037	< 0.01	1.005 ± 0.047
Na/Creatinine (μEq/mg)	15	163 ± 35	< 0.05	93 ± 13	< 0.05	163 ± 29	< 0.5	160 ± 36
$U_{PGE}V$ (ng kg^{-1} h^{-1})	5	0.50 ± 0.05	< 0.02	0.30 ± 0.05	< 0.001	0.77 ± 0.11	< 0.2	0.55 ± 0.23
$U_{PGF}V$ (ng kg^{-1} h^{-1})	5	0.43 ± 0.06	< 0.1	0.29 ± 0.06	< 0.05	0.54 ± 0.11	< 0.35	0.47 ± 0.11
BP (mm Hg)	15	89.18 ± 13.50	< 0.15	111.29 ± 14.60	< 0.3	98.17 ± 16.64	< 0.4	108.00 ± 22.67

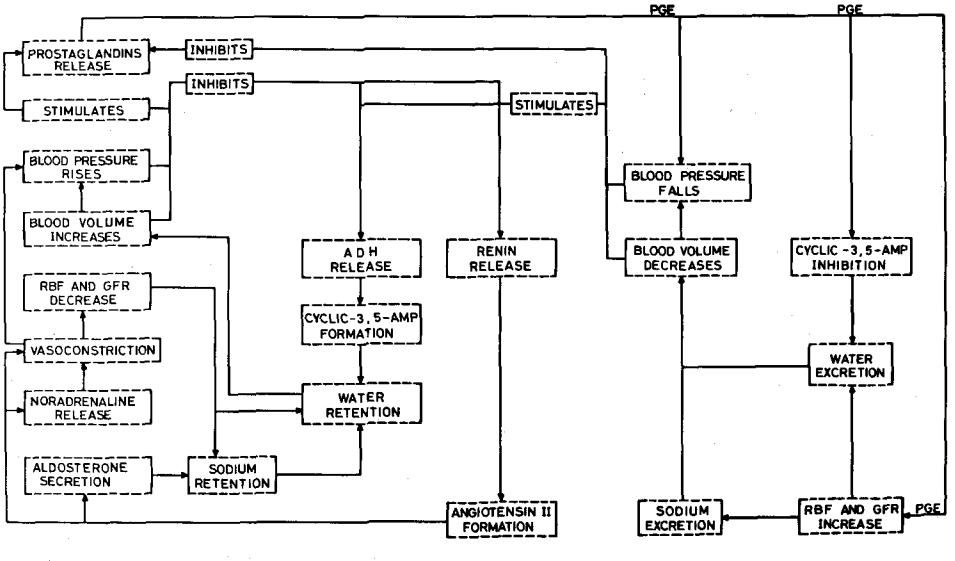

Fig. 2. Schematic portrayal of the possible homeostatic role of prostaglandins on blood pressure volume and sodium and water balance regulation.

and 0.01). A decrease in urinary PGEs concentration has also been observed (tables 1 and 2, figure 1).
These results provoked by the coadministration of aspirin could be reversed by the infusion of the synthetic PGA_2 (table 1 and figure 1).
Discussion. The data reported here confirm previous findings that aldosterone is able to inhibit (about 50%) the synthesis of PGs in experiments in the skin of the rat[12]. The same result have been obtained by using other anti-inflammatory steroids. In our experiments, aldosterone decreased sodium and increased potassium excretion resulting to a significant decrease of the index (urinary Na/K ratio) in both subgroups, of adrenalectomized and intact animals. These results were accompanied by a significant decrease in renomedullary PGEs release (tables and figure 1). The mechanism by which aldosterone (and other steroids) decreases the synthesis and/or release of PGs is not well-known, but among the suggestions offered is, that: it is a) to prevent the replacement of the synthetase enzyme, b) to interfere with the target organ, and c) to prevent the transport or the release of PG precursors as arachidonic acid[13,14]. Thus the antinatriuretic effect of aldosterone increases by his inhibitory effect on the natriuretic renomedullary PGEs synthesis and/or release. On the other hand, the natriuresis observed by many investigators after long-term administration of aldosterone could be explained by the release of PGEs following extracellular space expansion provoked by the retention of sodium[7,15,16]. The increase of the index (urinary Na/K ratio) following the coadministration of spironolactone observed in these experiments is well-known and the phenomenon could be related to the occupation of the mineralo-corticoid receptors by the substance and/or to the mediation (at least in part) by the simultaneous release of the potent natriuretic PGEs (tables and figure 1). This suggestion is supported by the results obtained when aspirin, a well-known inhibitor of PG synthesis and/or release, was coadministrated[17]. Thus, the index (urinary Na/K ratio) decreased, accompanied by a simultaneous decrease in PGEs concentration in the urine (forth subgroups, tables and figure 1), an effect which could be reversed by the infusion of the synthetic PGA_2 (table 1 and figure 1). The figure 2 shows schematically the possible feed-back between the natriuretic, diuretic and antihypertensive PGEs and their antagonists renin-angiotensin system, aldosterone, noradrenaline and antidiuretic hormone (ADH).

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The effect of methaqualone on prenatal development in the rat

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31 May 1977

Summary. Methaqualone treatment of pregnant rats in doses of 100–200 mg/kg day produces resorption and a series of anomalies whose incidence increases with the dose-level employed.

Methaqualone (2-methyl-3-orthotolyl-4-quinazolone) is a nonbarbiturate central nervous system (CNS) depressant which has become frequently abused and is presently highly sought after in the illicit drug market[2–4]. The widespread abuse of methaqualone has created a great deal of interest in, and research on its properties. Surprisingly, there is little information regarding the effect of methaqualone consumption during pregnancy[5,6]. In light of the evidence attesting to the growing number of individuals abusing methaqualone in nontherapeutic doses, we have investigated several dose-levels of methaqualone to assess the full spectrum of its effects on fetal development.
Material and methods. Pregnant Long Evans Hooded rats were administered, as a single daily s.c. injection in a propylene glycol suspension, 100, 125, 150 or 200 mg/kg methaqualone on days 8–15 of gestation or 115 mg/kg methaqualone on days 1–19 of gestation. If maternal deaths occurred, injections were continued until at least 5 mothers per group reached day 20 of gestation, except in the 115 mg/kg group where only 4 mothers were used and the infants were allowed to come to term. 5 females (normal control group) were not injected, but were weighed daily. 5 females (yoke control group) were administered daily s.c. injections of propylene glycol and were administered Purina Lab Chow in quantities just sufficient to maintain their weight at the mean level of the methaqualone groups. On day 20, these animals were sacrificed, their uterine horns exposed and the number of dead and living fetuses and resorption sites counted. The fetuses were weighed, examined for external defects, and their brains removed and weighed. For skeletal examination, the fetuses were placed in alcohol and stained with alizarine red S[7].

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