molecule should be taken into account, such as bulkiness, rigidity and the ability to form hydrogen bonds with the phosphate oxygen of the phospholipids.

The presence of a net charge on the membrane surface does not qualitatively change the action of tetracaine and hexobarbital, but influences the increase of resistance produced by a given dose of anesthetic (PS bilayers, Table I). A -158 mV surface potential has been reported for the PS membranes at pH 7.2[12] and can be assumed to be present at the surface of PS bilayers in the pH range of our experiments[14]. This negative surface potential could modify the concentration of the anesthetic in a region near to the interface. Hexobarbital, which dissociates as an anion, should be diluted and tetracaine, which dissociates as a cation, should be concentrated with respect to the bulk aqueous solution. In fact, hexobarbital at 0.1 mM increase 40 times the resistance of the neutral PE at pH 6.4 and only 28 times the resistance of the strongly charged PS at pH 6.4. The opposite is valid for tetracaine: the more dense the surface charge, the more effective the drug.

It can be stated that the concentration of the drug in the membrane phase is determined by the partition coefficient and the interfacial concentration. The latter depends on the sign and density of the surface charge of the membrane and on the sign of the ionized form of the drugs in solution. Both factors are affected by the dissociation of the drug. However, the effect on the partition coefficient is the most important, as can be seen in the experiments with tetracaine on the PS membrane, where the pH change influences only the dissociation of the drug and not the surface charge of the membrane. Tetracaine is more active at pH 8.4, when the partition coefficient is higher and the ionized fraction is lower.

At concentrations near 1 mM, both drugs caused a large decrease of the resistance values (below those of the intrinsic bilayers) and finally (over 2–3 mM) the films tended to break into the solution. The drop in resistance was probably due to coarse rearrangement of the double layer structure, such as local micellizations.

The molecular mechanism by which the presence of the anesthetic in the bilayer produces an increase of resistance, is still not understood. We suggest that the insertion of relatively rigid structures like the anesthetic molecules in the bilayer can reduce the fluidity of the first carbons of the fatty acids. It is well known that cholesterol, which decreases the fluidity of artificial membranes[16], also reduces their permeability[17]. Local anesthetics, which increase bilayer rigidity, may thereby also increase the bilayer resistance.

Riassunto. Viene studiato l'effetto della carica elettrica sull'interazione tra farmaci ad azione anestetica locale e fosfolipidi di membrana. Viene messa in evidenza la natura principalmente idrofobica di questa interazione.

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Modification of Diuresis in the Rat by Chlorpropamide, Glibenclamide and Tolbutamide

Oral hypoglycaemic drugs are of increasing importance in the management of maturity onset diabetes. Some of these drugs have been implicated in disorders of water and electrolyte balance. For instance several workers have reported an antidiuretic action of chlorpropamide in water loaded normal subjects[1,2] water loaded subjects with diabetes mellitus[3] and in patients with diabetes insipidus[1,2,4–6]. Several mechanisms have been suggested. One that has received most support is that of EARLEY[7] who suggested that chlorpropamide potentiated the effect of ADH. Such a mechanism would be expected to result in hyponatraemia and serum hypoosmolarity, symptoms demonstrated by Weissman et al. in five diabetics treated with chlorpropamide. A clinical survey showed that about 4% of patients treated with chlorpropamide developed these symptoms. WEISSMAN[8] suggested that in these 4% the cessation of ADH secretion in response to developing hyponatraemia was incomplete so allowing ADH potentiation to continue and result in more pronounced symptoms.

Further mechanistic investigations using experimental animal preparations have shown, for instance, that using the toad bladder[9] chlorpropamide has no effect alone, potentiates small ADH levels but has no such effect on large doses of ADH. Similar results have been obtained in vivo using a strain of rats with genetic hypothalamic diabetes insipidus[10]. The same study showed no antidiuretic effect of chlorpropamide in normal rats perhaps because these animals compensated for the increased effectiveness of their ADH by reducing ADH output. In both untreated rats with diabetes insipidus and normal rats sodium excretion was significantly increased during chlorpropamide treatment which in the diabetic rats led to an increase in urine volume. This effect of chlorpropamide may be separate from any effect on ADH.

Glibenclamide in contrast to chlorpropamide has been reported to enhance diuresis in normal subjects, patients with diabetes mellitus[3] and those with diabetes insipidus[11]. MOSES et al.[3] have suggested that this effect is due to the

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drug either increasing glomerular filtration rate or inhibiting sodium reabsorption in the proximal tubule. Either effect would increase both sodium and water delivery to the loop of Henlé, the sodium then being absorbed and the water excreted.

The purpose of the present study was to further investigate the effect of chlorpropamide in both normal and experimentally diabetic rats and to determine the extent to which glibenclamide's clinical effects could be duplicated in rats. Tolbutamide was also included in the study.

If chlorpropamide does act by potentiating ADH then its effect may best be demonstrated by acute tests of diuresis which allow less time for a compensatory reduction in ADH levels. Chronic administration might be expected to have little ADH mediated effect on the majority of rats. Both chronic and acute administration schedules were therefore used for both chlorporpamide and glibenclamide. Acute tests only were carried out using tolbutamide.

Methods. Male CFE rats 180–200 g were distributed into groups of 6 and assigned to drug or control treatment. Animals were starved 18–20 h before tests but allowed free access to water.

In acute tests drugs were suspended in 5% acacia and administered orally via stomach tube at the dose indicated in 50 ml/kg suspension. Control animals received 50 ml/kg of 5% acacia only. Each rat was then placed on a wire mesh platform in a large plastic funnel. Urine was collected for 3 h, volume being noted each hour. After 3 h determinations were made of urine Na^+ and K^+ (Eel flame photometer), osmolarity (Osmette) and pH.

Experimentally diabetic animals were obtained by dosing with streptozotocin (40 mg/kg i.v.) 7 days prior to the test. Only animals exhibiting glycosuria (Ames Clinistix) were considered diabetic.

Chronic dosing with chlorpropamide (50 mg/kg/day) and glibenclamide (5 mg/kg/day) was carried out for 7 days prior to the test, doses being administered orally via stomach tube in $^1/_2$ ml 5% acacia/rat/day. Control rats received medium only. The last dose was given 18 h before test at which time both control and drug treated groups received 50 ml/kg water load. Statistical significance was assessed using Students t-test a p-value of 0.05 or less being assumed to indicate significance.

Results. Urine volumes. Chlorpropamide (300 mg/kg) significantly increased the 1, 2 and 3 h cummulative urine volumes in normal rats. The increased values of the test group treated with 100 mg/kg chlorpropamide were not statistically significant (Figure A). Larger mean values than controls were also obtained from the diabetic groups treated with 100 and 300 mg/kg chlorpropamide but these differences were not significant (Figure D). The group treated chronically with 50 mg/kg/day chlorpropamide showed a significantly lower 1 h volume than their controls (Figure F).

Glibenclamide (5 mg/kg) like chlorpropamide significantly increased the 1, 2 and 3 h cummulative volumes in normal rats (Figure B). Mean volumes were also significantly increased in diabetic rats (Figure E). Again as with chlorpropamide, chronic treatment with glibenclamide resulted in reduced volumes being excreted, the reduction at 1 h being significant (Figure F). Acute tolbutamide 500 mg/kg reduced the volume excreted, the effect being significant at 1 h only (Figure C).

Sodium and potassium excretion. Acute chlorpropamide (300 mg/kg) significantly increased Na^+ excretion in both normal and diabetic rats, the percentage increase being greater than the increases in urine volumes in both cases. In the normal rats this was accompanied by a significant decrease in K^+ excretion, K^+ excretion by the diabetic rats receiving chlorpropamide remained similar to that of the control diabetic animals, (Table I). After chronic treatment the apparent decreases in Na^+ and K^+ excretion were not significant (Table II). Glibenclamide (5 mg/kg) increased Na^+ excretion significantly in normal rats. K^+ excretion was not significantly changed in these rats. No significant change was seen in the excretion of either ion in the diabetic groups treated with glibenclamide (Table I). Rats treated chronically with glibenclamide (5 mg/kg/day) had reduced mean Na^+ and K^+ excretion but only in the case of K^+ was the reduction shown to be statistically significant (Table II). Acute tolbutamide (500 mg/kg) significantly reduced K^+

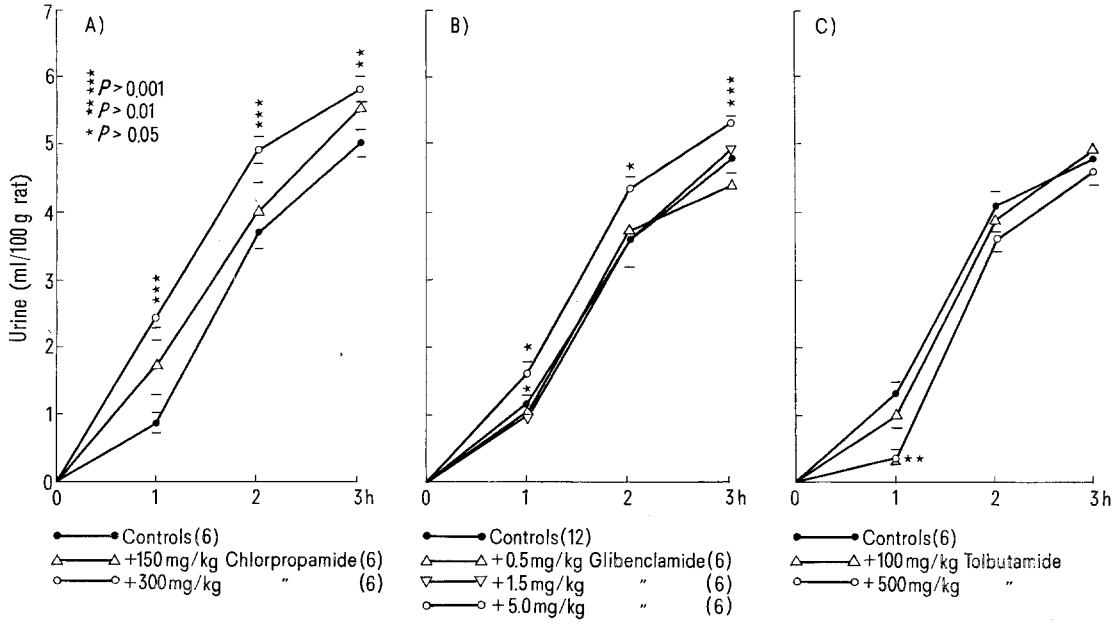

Fig. A–C. The effect of 3 orally administered hypoglycaemic drugs on the urine out put of normal rats. The abscissa represents hours from administration of either the water load alone (50 mg/kg) with or without drug.

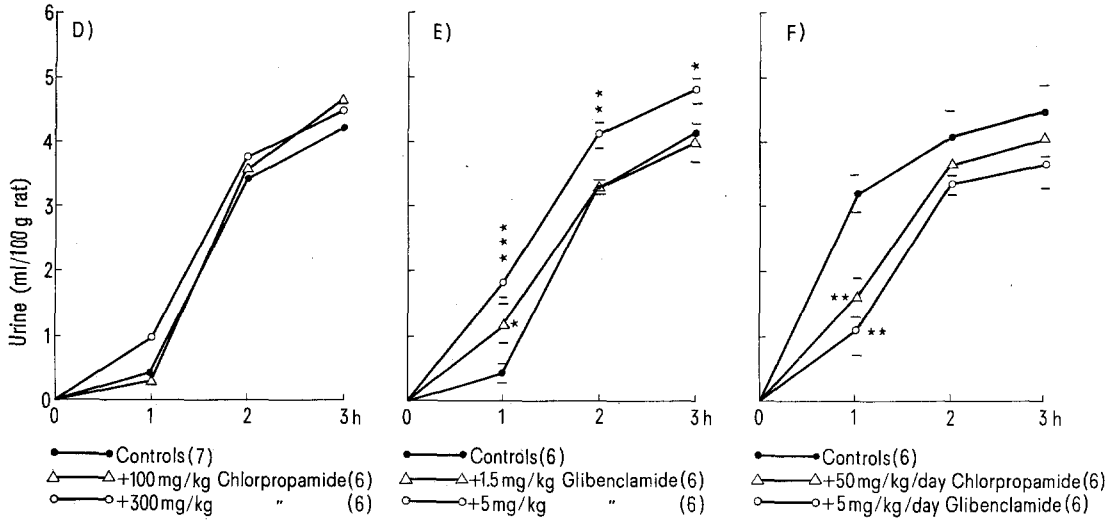

Fig. D and E. The effect of chlorpropamide and glibenclamide on the urine output of rats made diabetic with streptozotocin.
Fig. F, The effect of 'chronic' administration (daily dose for 7 days) of chlorpropamide and glibenclamide on the urine output of normal rats.

Table I. The Effect of acute oral administration of the 3 hypoglycaemic drugs on sodium and potassium excretion

		Dose (mg/kg)	μEq. excreted ± S.E. Na^+	K^+
Chlorpropamide	Normal rats	Controls (6)	26 ± 4	77 ± 8
		100 (6)	39 ± 11	66 ± 11
		300 (6)	82 ± 11[c]	54 ± 4[a]
	Diabetic rats	Controls (7)	8 ± 2	48 ± 6
		100 (6)	15 ± 6	47 ± 7
		300 (6)	56 ± 15[c]	48 ± 6
Glibenclamide	Normal rats	Controls (12)	26 ± 3	71 ± 9
		0.5 (6)	22 ± 11	54 ± 11
		1.5 (6)	35 ± 7	49 ± 8
		5.0 (6)	35 ± 2[a]	88 ± 2
	Diabetic rats	Controls (6)	7 ± 1	54 ± 6
		1.5 (6)	6 ± 2	49 ± 6
		5.0 (6)	6 ± 1	59 ± 6
Tolbutamide	Normal rats	Controls (6)	31 ± 5	68 ± 7
		100 (6)	20 ± 5	49 ± 9
		500 (6)	34 ± 6	34 ± 2[c]

Significance of difference from control group given by: [a] $p < 0.05$; [b] $p < 0.01$ and [c] $p < 0.001$. Numbers of animals per group are given in parentheses. S.E. denotes standard error of the mean.

Table II. The effect of chronic oral administration of the 3 hypoglycaemic drugs on sodium and potassium excretion

	μEq. excreted ± S.E. Na^+	K^+
Controls (6)	41 ± 13	88 ± 19
Chlorpropamide (50 mg/kg/day) (6)	21 ± 7	50 ± 3
Glibenclamide (5 mg/kg/day) (6)	20 ± 7	40 ± 10[a]

The last dose was given 18–20 h before water loading. Significance and number per group are indicated as in Table I.

excretion having little or no effect on Na^+ excretion (Table I).

If the diabetic control groups are compared to normal rats it can be seen that streptozotocin diabetes resulted in significantly reduced Na^+ excretion, no change being seen in K^+ excretion (Table I).

Osmolarity. The only changes in total urine osmolarity found to be significant were an increase in the diabetic controls compared to normal rats (from 645 ± 52 to 794 ± 84 μosmols/100 g body weight) and a decrease in the group chronically treated with glibenclamide compared to their controls (from 737 ± 72 to 505 ± 69 μosmols/100 g body weight).

Urinary pH. The only pH changes found to be significant were increases in the diabetic groups treated with both doses of chlorpropamide compared to the diabetic control group. The mean pH ± S.E. of the controls was 6.7 ± 0.1 and those of the groups treated with 100 and 300 mg/kg chlorpropamide were 7.7 ± 0.4 ($p < 0.05$) and 7.6 ± 0.2 ($p < 0.01$) respectively.

Discussion. The doses of the 3 hypoglycaemic drugs required to significantly affect diuresis are in the region of 50 × normal clinical doses. This relatively low potency is also indicated by the doses of chlorpropamide used by MOSES and MILLER[10]. An explanation for this may lie in the fact that diuretic drugs themselves are generally less potent in the rat. The pattern of changes in water diuresis caused by acute chlorpropamide was not indicative of ADH potentiation. Urine volume was increased significantly, the greatest increase being within the first hour of collection when ADH might have been expected to be available for potentiation. Maximal ADH levels in these animals were not expected since up until the time of test they were allowed free access to water. It therefore appears that only very small ADH levels are potentiated by chlorpropamide, larger but still submaximal levels not being potentiated. This is in agreement with the findings of MOSES and MILLER[10] whose chronic chlorpropamide dosed normal rats showed no antidiuresis although they also had free access to water and would therefore be expected to have submaximally effective blood levels of ADH.

The most striking effect of acute chlorpropamide on diuresis was the increase in Na^+ excretion (24 → 82 μEq/100 g at the 300 mg/kg dose in normal rats). This effect

was presumably the cause of the increase in urine volume. It was accompanied by a smaller but at the higher dose still significant reduction in K^+ output and therefore suggests an inhibition of the output or effectiveness of mineralcorticoids. Mineralcorticoid levels might be expected to be higher in the diabetic rats due to a probable reduction in blood volume caused by persistent glycosurea and hyperkalaemia due to cellular damage (β-cell destruction caused by such low doses of streptozotocin is a slow process and would be expected to be continuing after 7 days[12]. High mineralcorticoid levels are indicated by the fact that the $Na^+:K^+$ ratio was much reduced in the control diabetic animals compared to the normal controls, mean urinary pH also being lower but not significantly so. After the larger chlorpropamide dose the $Na^+:K^+$ ratio was greatly increased. pH values were also significantly increased both effects further indicating either an inhibition of mineralcorticoid effectiveness or output by chlorpropamide.

Water diuresis following chronic chlorpropamide dosing showed significantly reduced water output. This is the opposite of the acute response and probably represents a compensatory effect occuring at a time when blood chlorpropamide levels were relatively low (last dose of chlorpropamide was given 18 h before the test).

The diuretic effect of glibenclamide in both normal and diabetic rats is in agreement with the clinical findings[9,3] and the hypothesis that this drug increases sodium and water delivery to the loop of Henlé by either increasing glomerular filtration rate or inhibiting sodium reabsorption in the proximal tubule[3]. As with chlorpropamide the situation was reversed after chronic dosing presumably for the same reason, i.e. a compensatory effect occurring during relatively low blood levels of the drug. Acute tolbutamide in contrast to the other two agents reduced urine volume. The reduction in K^+ excretion was significant ($p < 0.001$) at the higher dose and since Na^+ excretion was relatively unaffected the $Na^+:K^+$ excretion ratio was significantly raised suggesting as wtih chlorpropamide mineralcorticoid antagonisms. Such an effect however could be expected to increase urinary volume and if it does occur then it may not be the only mechanisms by which tolbutamide affects the pattern of water diuresis.

The question as to whether chlorpropamide and possibly tolbutamide reduce mineralcorticoid activity by reducing output or inhibiting their effectiveness on the kidney could be resolved only by determining mineralcorticoid excretion under the influence of the drugs. Increased levels of the hormones would be expected, due to a compensatory increase in secretion if the effect of chlorpropamide is at the level of the kidney.

Zusammenfassung. Es wurden die Wirkungen von Chlorpropamid, Glibenclamid und Tolbutamid auf die Nieren wasserbelasteter normaler und diabetischer Ratten untersucht.

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Alcohol-Induced Pulmonary Changes in Rats

A relationship between excessive chronic alcohol intake and lung disease has been suspected for many years. Severe and sometimes fatal episodes of pneumonia, pulmonary emphysema, pulmonary fibrosis, and/or bronchiectasis are frequently associated with chronic alcoholism. Burch and DePasquale[1] suspected that these changes are due, at least in part, to direct damage of lung tissue by alcohol, and suggested the existence of an alcoholic lung disease. Recently Banner[2] studied the pulmonary function in 30 alcoholic patients and found that diffusion impairment and mild obstruction are characteristics of chronic alcoholism. The purpose of the present paper is to describe the effects of prolonged alcohol consumption on the morphology of rat lungs.

Materials and methods. 12 Wistar strain male rats (body weight 130–150 g) were allowed to drink only white rum (40% alcohol) for 4 months. 6 animals from the same stock were kept as controls and given no alcohol. All animals were fed on a balanced commercial rat food ad libitum. The daily amount of white rum consumed during the experiment was 4.9 ml/100 g of body weight.

Tissue samples of the lungs were minced, fixed in cold 1.5% glutaraldehyde (in 0.1 *M* cacodylate buffer, pH 7.4, containing 3% sucrose) for 2 h, and postfixed in buffered 1% osmium tetroxide for 1 h. The tissue blocks were dehydrated with acetone and embedded in araldite[3]. Thick sections used for light microscopy were stained with toluidine blue; ultrathin sections cut on a Porter Blum microtome were stained with uranyl acetate and lead citrate. Electron micrographs were taken on a AEI-EM6B electron microscope.

Results. Minor signs of chronic murine pneumonia were seen in both control and experimental animals. This will not be considered further. The lungs of the animals after long-term administration of alcohol showed a proliferation of alveolar cells and thickening of alveolar walls (Figure 1). The alveolar cells were found in increased number and size both as lining cells and free within the alveolar spaces. The intraalveolar cells were large mononuclear macrophages of various sizes, which tended to be ovoid in shape, and had a foamy appearance due to numerous intracytoplasmic vacuoles. Their cytoplasm contained also dark small round inclusions in the toluidine blue-stained thick sections. These free cells were not evenly distributed throughout the whole pulmonary tissue, but groups of alveoli were heavily affected, while others were empty. Hyperplasia and hypertrophy of granular (type 2) pneumocytes with a vesicular cytoplasm could be also seen where the foam cells were present. Similar pathological changes could not be observed in the lungs of control animals.

With electron microscopy, the changes consisted of hyperplasia and hypertrophy of type 2 pneumocytes, intraalveolar accumulation of macrophages and alveolar wall thickening due to increase in collagen content

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