l'expérimentation in vitro a été effectuée sur des coupes de cœur. Sur celles-ci, l'angiotensine ne provoque pas la libération de la noradrénaline[25] et les variations hémodynamiques sont évitées. Il est cependant possible de différencier les réductions d'accumulation tissulaire de noradrénaline provoquées par les variations hémodynamiques de celles qui sont secondaires à une inhibition du captage axonal membranaire. L'inhibition du captage accroît la quantité de médiateur sur les récepteurs adrénergiques; il en résulte une augmentation tissulaire des métabolites de la noradrénaline et, en particulier, des dérivés méthoxylés (IVERSEN et al.[45]), simultanée à la réduction de l'incorporation de noradrénaline, comme nous l'avons constaté avec la désipramine. Aucun des auteurs précédents n'a dosé les dérivés de la noradrénaline[46, 47].

En outre, nos expériences montrent l'absence d'inhibition par l'angiotensine des mono-amino-oxydases.

Ainsi, nos résultats montrent que l'angiotensine ne perturbe pas l'accumulation de noradrénaline au niveau du cœur, ce qui est en accord avec les résultats pharmacologiques ne montrant pas d'augmentation des effets presseurs de la noradrénaline exogène par le peptide.

Summary. The action of angiotensin II on cardiac uptake of norepinephrine was investigated in the rat in vivo and in vitro. In contrast to desipramine, neither infusion of subpressive (10 ng/kg/min) or pressive (50–150 ng/kg/min) amounts of angiotensin on intact and/or binephrectomized rats, nor incubation of cardiac slices with angiotensin II (10^{-5}; 10^{-9} *M*) impair the accumulation of tritiated norepinephrine and the level of metabolites. It is thus concluded that there is no inhibiting action of angiotensin II on the cardiac uptake of norepinephrine.

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The Effect of Oestradiol-17β on Uterine Adenosine Triphosphatase in the Rat

SKOU[1] has suggested that Mg^{2+}, Na^{+}, K^{+} stimulated adenosine triphosphatase (ATPase; E.C. 3.6.1.4 ATP phosphohydrolase) could be an essential mechanism by which cells maintain a low concentration of sodium. HALL[2] has demonstrated a decrease in uterine ATPase in mice during ovum implantation, and it has been suggested that alterations in activity of this enzyme could be related to the influx of water and sodium into the oestrous uterus[3]. The following is an extension of this work by examining the histochemical localization of ATPase in the uteri of oestrogen treated ovariectomized rats, and by measuring the levels of this enzyme in the microsomal and mitochondrial fractions of uterine homogenates.

Methods. White Wistar female adult rats weighing 220 ± 20 g were used. Ovariectomy was performed, and after 7 days oestradiol-17β (5 µg in 0.1 ml arachis oil/rat) or 0.1 ml oil was administered by s.c. injection to test and control animals respectively. After 23–24 h the rats were killed by cervical dislocation.

The uteri were then examined for ATPase activity using histochemical and quantitative chemical methods. For the histochemical study sections of uterus 10 µm thick were cut at − 20 °C and then stained for ATPase[4, 5].

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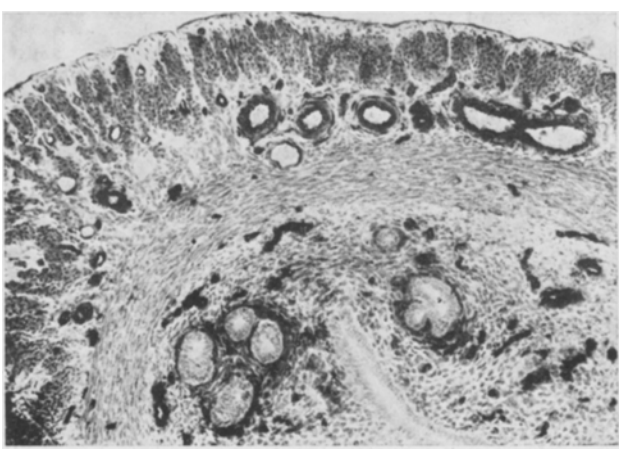

Fig. 1. Section of uterus of ovariectomized adult rat stained for ATPase activity. Black colouration indicates the presence of the enzyme. ATPase present in greater amounts in outer muscle layers, blood vessels and stromal connective tissue cells. × 86.

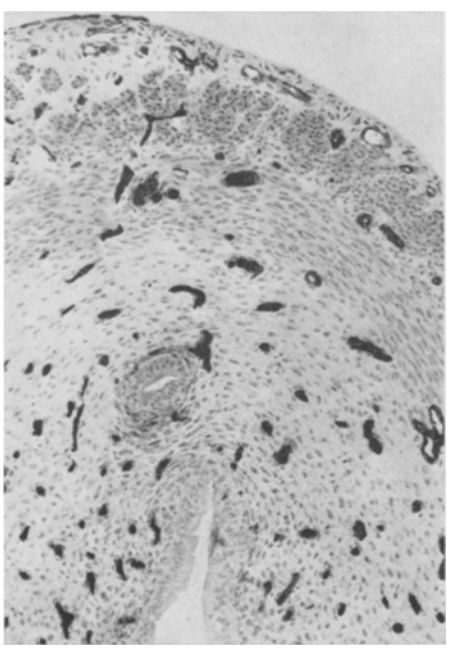

Fig. 2. Section of uterus from a rat treated with 5 µg of oestradiol-17β 24 h previously. Compared with Figures 1 and 3 there is much loss of ATPase in the outer muscle layers and persistance of activity in blood vessels. × 84.

Na^+, K^+, Mg^{++} ATPase activity of uterine mitochondria and microsomes from control and oestradiol treated rats

Preparation	Control			Test			Mean difference (control-test)	Significance of difference (*t*-test)
	Mean	No.[a]	Standard error	Mean	No.[a]	Standard error		
Mitochondria	3.52	7	0.256	2.28	7	0.325	1.24	$P<0.001$
Microsomes	6.74	7	0.892	3.73	7	0.350	3.01	$P<0.001$

Expressed as μmoles inorganic phosphate released (mg protein/5 min). Final concentrations in incubation mixture: *tris*, 50 m*M*, pH 7.3 $MgCl_2$ 3 m*M*; NaCl 90 m*M*; KCl 10 m*M* about 0.04 mg mitochondrial or microsomal protein in 0.1 ml of 0.25 *M* sucrose per ml. [a] Number of observations. Each observation based on a pooled specimen of 3 uteri.

Quantitative assays of ATPase were carried out by measuring the rate of release of inorganic phosphate from substrate (adenosine 5′-triphosphate-*tris* salt) at 37 °C[6]. The measurements were made on subcellular fractions prepared as follows: following blotting and weighing the uteri, they were homogenized in ice cold 0.25 *M* sucrose, 0.001 *M* *tris*, pH 7.3 (1 g/10 ml). The suspension was centrifuged at 900 × *g* for 12 min at 4 °C, and the pellet re-extracted. The combined supernatants were centrifuged at 12,000 × *g* for 20 min at 4 °C. The tan-coloured mitochondrial pellet was resuspended in the sucrose-*tris* solution in the proportion 1 g original tissue/10 ml solution. The mitochondria were centrifuged at 10,000 × *g* for 20 min. The supernatants were pooled and centrifuged at 36,000 × *g* for 2 h. The microsomal pellet was washed in the same medium.

Results. Widespread ATPase activity was demonstrated in the control rat uteri. The most intensely staining regions were the walls of the blood vessels in the myometrium and in the endometrial stroma. The myometrium also exhibited marked activity – particularly in the outer predominantly longitudinal muscle layer (Figure 1). Much ATPase activity was detected in the connective tissue cells of the endometrial stroma and this was usually increased in the region beneath the basal aspects of the columnar endometrial epithelium-lined diverticulae away from the main uterine lumen (Figures 1 and 3). There was only moderate enzymic reaction in the endometrial epithelium. Following oestradiol treatment, the uteri showed an overall loss of ATPase reaction-particularly in the outer muscle layer (Figure 2). The intense staining of the blood vessels persisted however (Figures 2 and 4). Another characteristic change was loss of the connective tissue activity in the region of the endometrial epithelium lining diverticulae of the central lumen (Figures 2 and 4). The endometrial epithelium itself was essentially unchanged in its staining reaction.

The Table shows a decrease in ATPase activity in both the microsomal and mitochondrial fractions in the uteri of oestrogen treated animals. The greatest drop in activity occurred in the microsomal fraction, but in both fractions the decrease was highly significant statistically ($P<0.001$).

Discussion. This decrease in ATPase activity is different from the overall stimulant effect of oestrogens on protein synthesis in the uterus[7–12]. The delay in the onset of

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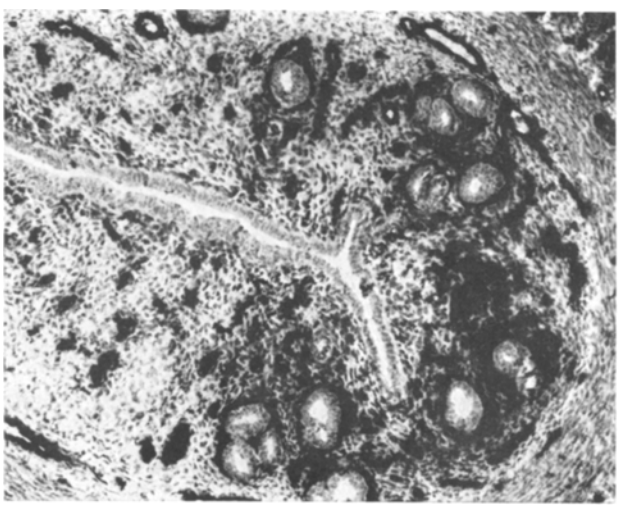

Fig. 3. Similar to Figure 1, showing a region of epithelial diverticulae from the main uterine lumen. Surrounding the diverticulae is marked stromal enzyme activity. × 85.

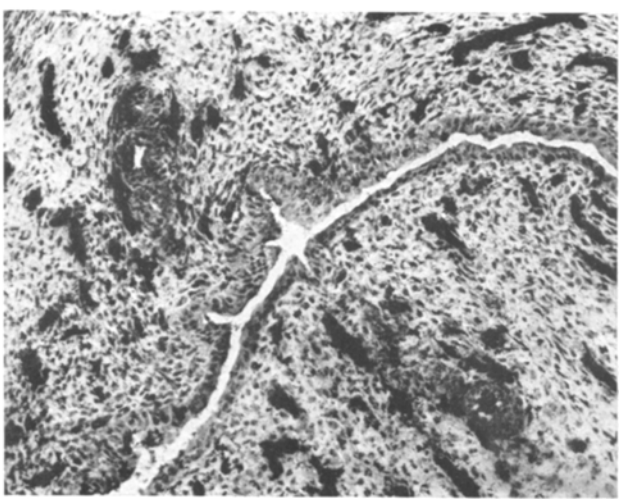

Fig. 4. Similar to Figure 2 showing no accentuation of connective tissue enzyme activity surrounding epithelial diverticula of the central uterine lumen. × 96.

ATPase loss suggests a decrease in synthesis in enzyme protein rather than a direct drug-enzyme interaction.

The histochemical demonstration of loss of ATPase in blood vessels, smooth muscle and stromal cells is more extensive than the changes observed by HALL[2,13] in pregnant mice, who observed complete loss of enzyme activity in the blood vessels of the decidua at the implantation site. HALL suggests that such loss is not due to progesterone alone but requires the presence of oestrogens. The results of the present experiments provide support for the possibility that oestrogens alone can produce this effect.

Zusammenfassung. ATPase-Aktivitätsmessungen im Uterus der Ratte nach 17β-Oestradiol ergeben ein relativ differenziertes Wirkmuster für dieses Steroid.

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A Linear Correlation Between the Amount of ^{3}H-Ouabain Administered Orally and that Absorbed by the Gastrointestinal Tract in Guinea-Pigs

In recent years, methods using radioactive isotopes have enabled more accurate and complete results to be obtained in studying the pharmacodynamics of cardiac glycosides than those previously achieved by indirect biological techniques[1]. However, the intestinal absorption of ouabain has not yet been thoroughly studied. In effect, the limited data reported in the literature on this subject are not in agreement, particularly as far as regularity of absorption is concerned[2–5]. This investigation was aimed at determining the amount of ouabain absorbed by the intestinal route and in particular at ascertaining whether there is any linear correlation between the dose administered orally and the amount of the drug absorbed. For this purpose, investigations were undertaken on conscions guinea-pigs.

Materials and methods. The tests carried out to check the purity of ^{3}H-ouabain[6], the stability of radioactivity in contact with biological fluids and tissue homogenates, and the standardization of the analytical method used for quantitative determination of the drug are described in detail in other papers[7,8]. Tests were performed on 63 conscious guinea-pigs which had fasted for 24 h. The animals were divided into 3 groups and treated orally with ouabain at doses of 250, 500 and 1000 µg/kg. 21 of them were killed after 1 h, 21 after 5 h and 21 after 15 h, providing a total of 9 groups of 7 guinea-pigs each. The glycoside solution utilized had an ouabain concentration of 50 µg/ml and a labelled product activity of 2.84×10^6 dpm/ml. Ouabain radioactivity was measured in the heart, liver, kidneys, muscle, carcass, intestine and stomach, intestine contents, spleen, bile, blood and urine. Small portions of each organ were dissolved in Packard Soluene TM 100. The solution thus obtained was added to the scintillating solution and counted in a Packard Type 3320 Liquid Scintillation Spectrometer for 50 min.

In order to calculate the percentage rate of ^{3}H-ouabain intestinal absorption, all the activities found in all organs and biological fluids examined were added together, except the intestine contents. The absorption percentage rate was calculated from the sum thus obtained and the total activity administered orally. For this calculation, the blood mass and the muscular mass of the guinea-pigs were assumed to amount to 10% and 40% of body weight respectively. The carcass weight was calculated from the difference between the total weight of the guinea-pig and the sum of weights of all the organs and biological fluids examined.

Results and discussion. ^{3}H-ouabain intestinal absorption in guinea-pigs (Table) amounted to around 3.6%, 6–7% and 9.5% after 1, 5 and 15 h respectively. In practice, these values were constant at each of the doses administered, amounting to 250, 500 and 1000 µg/kg respectively. This allowed the linear equations which relate the dose

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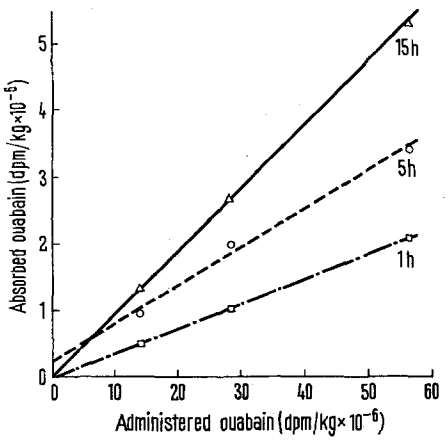

Linear correlation between ^{3}H-ouabain orally administered and ^{3}H-ouabain absorbed by the gastro-intestinal tract in guinea-pigs. The straight-line equations are calculated by the straight-line regression method. They are respectively:
After 1 h: ^{3}H-ouabain absorbed (dpm/kg) = — 40,494 + 0.0373 × ^{3}H-ouabain administered (dpm/kg) r = 1.000.
After 5 h: ^{3}H-ouabain absorbed (dpm/kg) = 226,000 + 0.0574 × ^{3}H-ouabain administered (dpm/kg) r = 0.9980.
After 15 h: ^{3}H-ouabain absorbed (dpm/kg) = 27,000 + 0.0937 × ^{3}H-ouabain administered (dpm/kg) r = 1.0000.