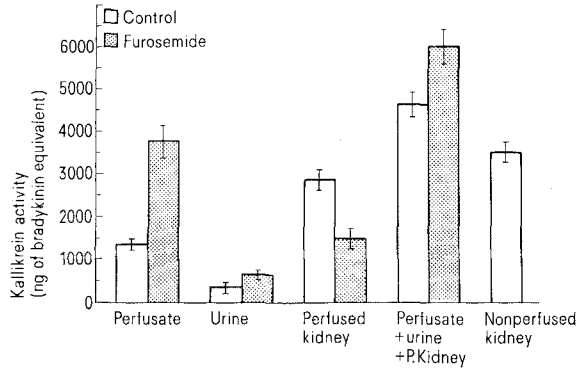
was continually monitored by a photoelectric drop counter (Grass Model PTTI). The measurements were recorded on a Grass polygraph. A single-pass perfusion system was used and was set up in a similar way as have been described previously[5].

The perfusion medium was bicarbonate Krebs-Henseleit solution (Na^+, 126 mM; K^+, 6.0 mM; Ca^{++}, 3.3 mM; Mg^{++}, 1.1 mM) modified by addition of 15 g/l bovine albumin (Calbiochem); 35 g/l Dextran 70,000 MW (Sigma Chemical Co.); 2 mM Na-pyruvate, 6 mM glucose, 6 mM urea and 10 mg/l creatinine. The solution was constantly equilibrated with a mixture of O_2/CO_2 (95/5, respectively)[5]. After equilibration, the pH ranged from 7.40 to 7.45.

To investigate the effects of furosemide, 20 mg/l of Laxur® (Hoechst AG) were added to the modified Krebs-Henseleit solution after the removal of the blood from the kidneys. Glomerular filtration rate was determined by the creatinine clearance using the method described by Folin and Wu[6].

To determine kallikrein activity, samples of renal perfusion outflow were taken every 15 min for 60 min, while urine samples were collected every 30 min for 60 min. Kallikrein activity was measured by methods previously described in perfusion outflow, urine samples[5] and renal tissue[7].

Results and discussion. Mean creatinine clearance in control kidneys was 0.57 ± 0.02 (SD) ml/min and 0.27 ± 0.01 in furosemide-treated kidneys. These values are in agreement with previous reports of others[8] and indicate adequate functioning of the kidneys. No significant changes in the vascular resistance were observed either in the control or in the furosemide perfused kidneys. Control and furosemide data are presented in the Figure.



Control and furosemide experiments. The height of the column represents the mean value for each group of samples; the bars indicate SD.

Kallikrein activity of the furosemide perfusate was significantly ($p < 0.001$) greater than the activity of the control perfusate. A significant increase is already observable even in the samples collected at the end of the first 15 min period. The highest concentration was obtained in the second sample and from then on it keeps constant. The difference between the urine samples were not statistically significant ($p < 0.01$). Kallikrein activity of the furosemide-treated kidney was significantly ($p < 0.001$) lower than of the control perfused kidney. The sum of kallikrein activity found in perfusate, urine and kidney of the furosemide experiments was significantly greater ($p < 0.01$) than the sum of the activities in control experiments.

The kallikrein activity of non-perfused kidneys was significantly lower ($p < 0.01$) than the total kallikrein activity (i.e. perfusate + urine + kidney) found in control perfused kidneys.

The significantly higher total kallikrein activity found in control perfused kidneys, compared to non-perfused kidneys indicates that perfusion of the kidneys with kallikrein-free medium activates some kallikrein precursor or/and stimulate synthesis and release of renal kallikrein. Furosemide intensifies further these effects, as shown by the significant difference between furosemide-treated and control perfused kidneys. Importantly, the effects of both perfusion and furosemide result mainly in a large increase of kallikrein activity in the perfusate. These findings strongly support the notion that renal kallikrein can be released to the blood stream in the physiological condition, and they therefore strengthen the possibility of a systemic role played by renal kallikrein.

Experiments on the effect of several factors including furosemide which increase diuresis-natriuresis upon renal and urinary kallikreins in normal rats[9] and the ability of rat kidneys slices to synthetize kallikrein[10], give support to the concept that furosemide many stimulate the synthesis of the renal enzymes. The amount of kallikrein excreted in the urine, under furosemide was slightly higher than the control, but statistically not significant.

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Changes in Heart Rate Levels During Avoidance Conditioning in the Rabbit

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Summary. During avoidance conditioning heart rate levels tend to increase or to decrease according to their initial values and these changes are not related to learning or performance of the task.

The general level of activation of an animal is usually indicated by several physiological measures such as the heart rate (HR) and the electroencephalographical activity (EEG)[2]. For instance, in the rat, it has been reported that HR levels are monotonically related to hours of water deprivation[3], while performance and deprivation

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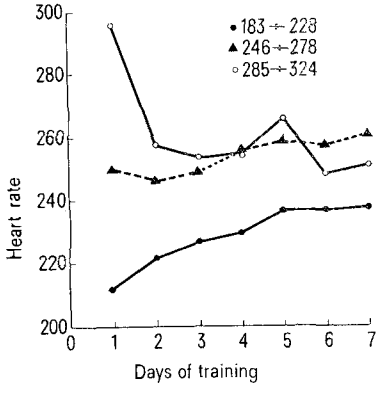
Heart rate levels during training

Exp. No.	I.	Max	Min	Max–Min	Cr. Day	Day 7	F. Day
17	183	255 (9)	180 (1,2)	75	237 (5)	205	198 (10)
53	195	252 (8)	171 (4)	81	216 (6)	230	240 (9)
38	195	288 (6)	195 (1)	93	195 (1)	275	275 (7)
22	210	264 (7)	198 (1)	66	–	264	264 (7)
27	219	273 (10)	215 (1)	58	–	264	246 (11)
44	222	284 (3)	204 (1)	80	240 (4)	228	240 (8)
28	222	285 (9)	222 (1,2)	63	–	256	258 (10)
15	225	294 (10)	195 (1)	99	264 (5)	288	294 (10)
19	228	270 (2,4)	198 (1)	72	–	252	252 (7)
36	285	294 (1)	220 (6)	74	–	232	232 (7)
51	300	312 (1)	246 (6)	66	258 (4)	258	264 (9)
41	306	306 (1)	228 (2)	78	252 (5)	264	264 (10)
49	324	330 (1)	234 (2,3)	96	288 (5)	288	246 (10)

Abbreviations. Exp. No., experiment number; I, initial HR values; Max, maximal HR values recorded during training (for samples collection see Figure); *Min, minimal HR values recorded during training; Max–Min, maximal variation in HR values for each subject;* Cr. Day, first sample of HR recorded on criterion day; Day 7, HR values recorded before trial 1 on day 7 of training; F. Day, HR values on last day of training. Numbers in parenthesis: day of training in which the value was recorded. Top of the Table: rabbits with initial HR between 183 and 228; bottom: initial HR between 285 and 324. Experiment No. 38 was not included in the results of the Figure since the animal reached the criterion of learning on day 1 of training. Once the rabbits reached the criterion of learning, the performance oscillated between 70 and 95% of avoidances.

times show an inverse U relation[4]. In rabbits not accustomed to a new environment, HR levels oscillate and EEG and behavioral aspects of sleep do not appear; a few hours later, when adaptation has occurred, HR levels are reduced and periods of sleep can be recorded[5]. In the present experiments HR levels have been studied in the rabbit during avoidance conditioning.

Methods. Female European rabbits (*Oryctolagus cuniculus*) carrying EEG, electromyographical activity (EMG) and HR implanted electrodes[5] were trained to avoid an electric shock (UCS) to the left foot by moving the left hind limb in response to a 4 sec sound (CS). Increase in EMG activity of biceps femoris muscle interrupted CS and prevented UCS. Each daily session of training consisted of 30 stimulus presentations delivered at variable intervals of 1–2 min when the animal was not moving. The criterion of learning was established at 9 avoidance responses in 10 successive trials; rabbits reaching criterion received additional training sessions. 11 out of 26 animals were unable to meet the learning criterion.



Time course of heart rate changes during 7 days of avoidance conditioning. For every training session, 4 samples of 10 sec HR tracings, recorded in absence of rabbit movements, were analyzed during the following periods: before trial 1, between trial 9 and 10, between trial 19 and 20, after trial 30. Filled circles, initial HR values (first HR samples recorded on day 1 before trial 1) between 183 and 228 beats/minute; N = 8 Ss, learning criterion reached by 4 of them. Filled triangles, initial HR values between 246 and 278; N = 13 Ss, 7 of which reached learning criterion. Open circles, initial HR values between 285 and 324; N = 4,3 of which reached learning criterion.

Results and discussion. Training situation affected HR levels according to the initial values of HR (first value recorded before trial 1 on the first day of training), and no relationship was found to learning or performance. In one group of subjects (Ss) with low initial values (183 to 228), HR increased from session to session during training (Figure). The analysis of variance applied to data obtained during the first 7 days revealed that this increase was significant ($F = 5.2$; $df = 6/271$; $p < 0.01$). The Table shows that minimal HR was recorded on day 1 in 7 out of 8 Ss. In one of these animals (No. 38, Table), learning criterion was reached on day one, but HR continued to increase on the following days during additional training. In another group of Ss (initial HR values between 285 and 324) HR decreased significantly during training ($F = 10.6$; $df = 6/105$; $p < 0.001$). The Table shows that the maximal frequency was recorded on day 1 in all Ss. Finally in a group of Ss with initial HR values between 246 and 278, no clear tendency was recorded during training. Except in one experiment (No. 17, Table) no U curve changes in HR were recorded during training both in learning and non-learning animals.

Results suggest that, in the rabbit, during avoidance conditioning, changes in HR levels cannot be related either to learning or to performance of the task, but depend upon initial HR values.

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