pension in saline of red cells thoroughly washed in saline were added to 1 volume of a 200 mg/ml solution in saline of cephalothin (Keflin Lilly) (final concentration 40 mg/ml), the mixture was incubated in a waterbath at 37 °C for 3 h and gently mixed approximately every 30 min. The concentration of cephalothin solution, the drug/red cells ratio and the incubation time and temperature were adopted after they had proved to be optimal in a series of preliminary experiments. At the end of the incubation period, the red cells were repeatedly washed with a large volume of saline until the supernatant was completely free from hemoglobin. The in vitro lysis tests were carried on as previously described[4], and the Coombs tests were performed with different anti-γ and anti-non-γ reagents.

In addition to a positive non-γ (Hyland) Coombs test, as observed by MOLTHAN et al.[1], cephalothin-treated red cells give a positive Ham test: i.e. they lyse in slightly acidified fresh compatible normal serum (pH 6.5), while lysis does not appear if the above medium is previously heated at 56 °C for 30 min to destroy complement. Depending upon the different normal sera used, a varying susceptibility to lysis of the same altered red cells was observed: likewise, altered red cells from different healthy donors underwent different degrees of lysis when incubated in the same normal serum.

Cephalothin-treated red cells also give a positive cold-antibody hemolysis test. They are thus PNH-like, in that they behave in certain in vitro lysis tests in the same way as do the red cells of paroxysmal nocturnal hemoglobinuria (PNH). Cephalothin, a sodium salt of 7-(thiophene-2-acetamido) cephalosporanic acid, resembles certain chemically-different substances (proteolytic enzymes, sulphydryl compounds) in its capacity to cause a PNH-like lesion in the normal red cell membrane. The mechanism by which different substances can render normal red cells similar in some respects to PNH-cells is unknown. However, cephalothin seems to alter the cell membrane in a new way, for cephalothin-treated red cells react with antiglobulin serum while cells treated with proteolytic enzymes or sulphydryl compounds do not. This observation strongly suggests that this type of PNH-like cell is produced by an alteration to some of the proteins of the cell membrane and supports the hypothesis that the defect which occurs spontaneously in PNH is located in the protein moiety of the red cell stroma[5,6].

Riassunto. Emazie umane normali trattate con cefalotina, in opportune condizioni sperimentali, si comportano in alcuni test di emolisi in vitro (test di emolisi acida, test di sensibilità agli isoanticorpi freddi) in modo simile alle emazie della emoglobinuria parossistica notturna (EPN) (emazie simil-EPN). A differenza di altri tipi di emazie simil-EPN, queste emazie danno anche un test di Coombs diretto positivo, di tipo non γ. Viene avanzata l'ipotesi che la lesione responsabile del comportamento simil-EPN di queste emazie sia da ricercare in una alterazione delle proteine stromali.

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Urinary Output of Rats in Response to Subcutaneous Injections of Adrenaline

The effect of various doses of adrenaline on the urinary output of water and electrolytes in rats has been confusing. Early workers[1–6] found that injections of adrenaline in the range of 32–100 μg/100 g body weight caused diuresis and natriuresis in rats and concluded that these were the only effects of injected adrenaline. However, LOCKETT and her co-workers[7–9] further investigated these effects in rats, paying special attention to threshold effects. They used doses much smaller than those used previously. Threshold doses of 2.5 μg adrenaline/100 g body weight given s.c. up to 25 μg/100 g body weight, were found to be antidiuretic and sodium retaining. This antidiuresis was not affected by adrenalectomy, by partial hepatectomy, by removal of the posterior pituitary gland or by section of the afferent nerves from the injection site. They further showed that neither the systemic mean arterial pressure nor the renal clearance of inulin were affected by these doses. It was also demonstrated that doses ranging from 50–200 μg/100 g body weight caused a diuresis. However, they did not indicate whether there was an increase in sodium excretion. In this communication, experimental evidence is presented in an attempt to correlate the renal effects of adrenaline that have been reported.

Four separate groups of Wistar rats were used. They were injected s.c. with adrenaline in doses ranging from 2.5–40.0 μg/100 g body weight after an oral water load and observed over the following hour. The experimental technique has been previously described[10]. Each rat acted as its own control in the cross-over tests. The Table shows the results of the experiments, each carried out separately. Experiments 1 and 2 showed quite clearly that with a dose of 2.5 μg/100 g body weight, there was an antidiuresis as well as sodium retention. These effects were maximal with 5.0 μg/100 g body weight ($P < 0.01$

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Effect of adrenaline on normal rats

Experiment No.	No. of rats used	Body weight (g)	s.c. injection of adrenaline	Urinary excretion/100 g rat/h			
				Volume (ml)	Sodium (μ-Equiv.)	Potassium (μ-Equiv.)	Na:K
1	12	182 ± 1.88	Control	1.89 ± 0.09	14.8 ± 1.70	23.1 ± 2.65	0.66 ± 0.03
			2.5 μg%	1.31 ± 0.12[b]	10.4 ± 2.03[a]	14.1 ± 2.79[b]	1.08 ± 0.41
			5.0 μg%	1.30 ± 0.15[b]	9.1 ± 1.20[b]	12.5 ± 2.64[b]	1.16 ± 0.32
			10.0 μg%	1.37 ± 0.15[b]	11.9 ± 2.51	9.4 ± 2.12[c]	1.62 ± 0.41[a]
			20.0 μg%	1.54 ± 0.12[a]	18.5 ± 2.86	11.9 ± 1.94[c]	2.02 ± 0.44[a]
			40.0 μg%	1.80 ± 0.09	37.0 ± 4.27[c]	9.8 ± 1.53[c]	4.68 ± 0.96[b]
2	12	174 ± 1.43	Control	2.11 ± 0.13	18.9 ± 1.98	27.1 ± 2.22	0.70 ± 0.05
			2.5 μg%	1.66 ± 0.17[a]	12.2 ± 2.29[a]	14.7 ± 3.21[c]	0.89 ± 0.15
			5.0 μg%	1.28 ± 0.09[c]	8.7 ± 1.23[c]	11.2 ± 3.43[c]	1.13 ± 0.19
			10.0 μg%	1.57 ± 0.18[b]	8.9 ± 1.35[b]	9.2 ± 2.28[c]	1.38 ± 0.28[a]
			20.0 μg%	1.98 ± 0.21	23.2 ± 4.57	13.0 ± 3.01[c]	2.29 ± 0.56[a]
			40.0 μg%	2.44 ± 0.19	34.6 ± 4.71[b]	13.9 ± 2.91[b]	3.15 ± 0.51[c]
3	12	184 ± 2.17	Control	2.03 ± 0.09	16.0 ± 1.68	24.0 ± 1.50	0.66 ± 0.05
			5.0 μg%	1.38 ± 0.13[c]	9.5 ± 1.56[b]	12.6 ± 1.63[c]	0.79 ± 0.10
			40.0 μg%	1.74 ± 0.10[a]	42.0 ± 4.84[c]	15.6 ± 3.31[a]	3.71 ± 0.64[c]
4	11	175 ± 1.96	Control	2.18 ± 0.15	17.2 ± 1.87	25.3 ± 4.23	0.77 ± 0.10
			5.0 μg%	1.29 ± 0.17[c]	8.1 ± 1.29[b]	8.4 ± 1.48[b]	1.08 ± 0.16
			40.0 μg%	1.95 ± 0.17	40.2 ± 5.93[b]	10.5 ± 1.44[a]	4.18 ± 0.59[c]

The values shown are means ± standard error. % = per 100 g body weight. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.

for water and sodium in experiment 1 and $P < 0.001$ for both in experiment 2). With increasing doses, water output approached that of the controls. At 40.0 μg/100 g body weight, water output was almost similar to the control value in experiment 1 and was greater, though not significant, in experiment 2. Sodium output was converted from a retention to an increased extrusion as doses of adrenaline were increased. At 40.0 μg/100 g body weight, there was a significant natriuresis ($P < 0.001$ in experiment 1 and $P < 0.01$ in experiment 2). Potassium output was significantly depressed throughout the dose range used in both experiments and this resulted in significant increases in the Na:K ratio. Experiments 3 and 4 in which only 2 doses within the range were used, confirmed the observations on the first 2 experiments.

Although doses greater than 40.0 μg/100 g body weight were not used, these observations suggest that low doses of adrenaline injected s.c. in rats cause antidiuresis with sodium retention which are maximal with a dose of 5.0 μg/100 g body weight. With increasing doses the antidiuretic effect is lost and the diuretic phase sets in (seen in experiment 2, though insignificant at this stage). This observation is in full agreement with and confirms the findings of LOCKETT et al.[7-9]. The finding that there is significant natriuresis with increasing doses, extends their findings and confirms those made by earlier workers[1-6]. WINTON[11] using isolated dog kidneys observed a diuresis with his i.v. infusions of adrenaline. With higher doses, an antidiuresis occurred. He concluded that the first effect was caused by afferent glomerular artery vasodilation accompanied by efferent artery vasoconstriction thus causing increased glomerular filtration. The second effect was attributed to an intense local vasoconstriction of both afferent and efferent glomerular vessels. The diuretic phase in the intact animal can also be contributed to by (a) a rise of blood pressure[12], (b) the possibility of adrenaline blocking the release of antidiuretic hormone from the neurohypophysis[13,14] and (c) a sympathetic nervous mechanism[12].

It would therefore appear from past evidence and that presented in this report, that the renal responses in rats to adrenaline are dose dependent. In the low range, threshold doses and above, cause antidiuresis and sodium retention, due possibly to an effect on tubular reabsorption[9]. Higher doses cause a diuresis and natriuresis by an effect on glomerular arteries, possibly aided by the factors previously mentioned. The effects on tubular reabsorption exhibited by lower doses would be overcome by these higher doses. Doses much higher still would cause antidiuresis again due to intense vasoconstriction of afferent and efferent glomerular arteries[15].

Zusammenfassung. Es werden der Effekt einer s.c. Injektion von Adrenalin auf die renale Ausscheidung von Wasser und Elektrolyten bei Ratten sowie Versuche über die Korrelation verschiedener Dosis abhängiger, renaler Adrenalin-Wirkungen beschrieben.

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