Table II. Unilateral limb ischemia: The effect of drugs on euthyroid and hyperthyroid rats

Treatment	Euthyroid			Hyperthyroid			
	No. of rats	Survival time (h)	Mortality (%)	No. of rats	Survival time (h)	Mortality (%)	Significance
Saline	14	[a]	0	11	1.88 ± 0.41	100	
Reserpine	12	[a]	0	13	1.56 ± 0.36	100	$P > 0.01$
Phenoxybenzamine	12	[a]	0	15	5.82 ± 1.12	80[b]	$P < 0.01$

[a] Lived 7 days, then were sacrificed. [b] 3 of 15 lived 7 days and were sacrificed.

Table III. Parameters obtained (with the exception of heart weight) just prior to tourniquet application

Groups	No. of rats	Total body oxygen consumption (ml/h/100 g body wt.)	Heart weight (mg/100 g body wt.)	Heart rate (min)	Mean blood pressure (mm Hg)
1. Intact (euthyroid)	10	238 ± 21	362 ± 28	322 ± 46	112 ± 14
2. Intact and reserpine	8	222 ± 24	358 ± 36	304 ± 38	119 ± 18
3. Intact and phenoxybenzamine	8	202 ± 32	365 ± 23	316 ± 28	116 ± 11
4. Hyperthyroid	10	530 ± 64	602 ± 83	596 ± 88	228 ± 38
5. Hyperthyroid and reserpine	9	521 ± 49	638 ± 65	518 ± 72	184 ± 28
6. Hyperthyroid and phenoxybenzamine	10	436 ± 56[a]	618 ± 76	564 ± 76	206 ± 38

[a] $p < 0.05$ between groups 4 and 5.

thyroid hormone sensitizing the tissues to endogenous or catecholamines. If this were true we would observe some beneficial effect in tourniquet subjected hyperthyroid animals after reserpinization when the noradrenalin content of the heart was depleted. But, in fact, reserpine pretreatment does not change the survival time at all. Therefore, neither by increasing nor decreasing the endogenous catecholamine content are we able to alter the animals' susceptibility to shock.

Although the tachycardia of hyperthyroidism appears to be mediated by a hyperactive sympathetic system, metabolic effects do not seem to be so related; reserpinization of hyperthyroid animals does not change oxygen consumption (Table III) or cardiac output[7].

The use of a unilaterial tourniquet (Table II) which is not lethal to euthyroid rats[8], kills hyperthyroid rats within 2 h. Reserpine pretreatment again had no effect, however phenoxybenzamine not only increased the overall survival time but some of the rats survived as long as seven days, at which time they were killed.

The main beneficial effects of phenoxybenzamine is due to the blocking of peripheral constriction of circulating sympathomimetics thus enhancing peripheral blood flow[9]. We also observed a decreased total body oxygen consumption (Table III). Because the reflexes involving the heart are intact after α-adrenergic blockade[10], the lengthened survival time was due to improved peripheral blood flow and possibly a less demanding tissue oxygen utilization.

We conclude therefore, that the critical factor in the development of tourniquet shock of hyperthyroid rats is decreased peripheral efficiency rather than myocardial failure.

Zusammenfassung. Die Veränderung des endogenen Catecholamin-Gehaltes des Herzens hyperthyroider Ratten hebt die Schockempfindlichkeit der Tiere nicht auf.

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Effect of Phenformin and Chlorpropamide on Renin Activity in the Rat

Several drugs have been added during the last decade to the treatment of diabetes insipidus (DI), in addition to the substitution treatment with vasopressin. Chlorothiazide and other diuretics were introduced with considerable clinical success[1,2]. However, no satisfactory explanation for the mechanism of action of the benzothiadiazines in DI has been suggested. We have previously reported that thiazides had an antidipsic effect when applied to nephrectomized rats or following implantation in the hypothalamus[3,4]. The introduction of antihyperglycemic drugs, chlorpropamide and metformin, to the treatment of diabetes insipidus[5,6] presented another challenge as to the mechanism of action of the various unrelated drugs in this disease.

Recently BROWN et al.[7] have suggested a new and interesting hypothesis for the antidiuretic effect of thiazides in DI. The crucial factor according to this hypothesis is an increase in plasma renin activity and hence – angiotensin. This would then reduce urine volume and, secondarily, reduce the thirst, a remarkable feature in this disease.

This hypothesis could also explain the beneficial effect of a sodium-poor diet in DI: such a diet would maintain the elevated plasma renin levels caused by the drug[7]. BROWN et al.[7] also presented data on plasma renin activity in patients treated with thiazides, which supported the hypothesis.

It seemed to us that this hypothesis could be further generalized if the non-natriuretic drugs, such as antihyperglycemic compounds, which are effective in reducing urine flow in DI, could also increase plasma renin activity.

The experiments were performed in male rats of the Hebrew University strain, weighing 250–300 g. In the first experiment we have injected into rats the biguanide drug phenformin (kindly supplied by Assia Pharmaceuticals, Tel-Aviv), 25 mg/kg s.c. and 75 mg/kg i.p. The control rats were injected with equivalent volumes of saline (0.9% NaCl). The rats were given food and water ad libitum overnight. On the following day blood was collected and the kidneys were removed to study renin activity. The method of BOUCHER et al.[8] was used, incubating the samples with excess substrate (prepared from plasma of nephrectomized rats). The kidneys were subjected to 3 cycles of freezing and thawing before testing for renin activity (to release or expose bound renin). The results are shown in Table I: phenformin resulted in a fall, rather than an increase of plasma renin activity; no significant change was observed in kidney renin activity.

The second experiment was performed with chlorpropamide (kindly supplied by Charles Pfizer & Co., Inc., New York). The drug was dissolved in ethanol and the control rats were injected with equivalent volumes of ethanol. Chlorpropamide was injected s.c. (100 mg/kg) and the same procedure was followed as with phenformin. The results are given in Table II. The effect of chlorpropamide resembles that of phenformin, i.e. a decrease in plasma renin activity was observed and no significant change in kidney renin activity.

Thus the hypothesis of BROWN et al.[7] for the action of thiazides in DI through increased plasma renin activity cannot be extended to the anti-hyperglycemic drugs. The mechanism of the reduction of plasma renin activity by the antihyperglycemic drugs is not clear. However, this finding corroborates previous results from our laboratory: 1. The antihyperglycemic drugs were found to be antidipsic in the rat[9,10], and 2. Renin and angiotensin stimulate water intake in the same species[11,12]. However, the antidiuretic effect of these drugs in DI is not thereby explained. Experiments in progress now on the effect of these drugs on transport in amphibian skin may lead to further understanding.

The dipsogenic effect of renin requires also further elucidation of the beneficial effect of the diuretics in DI[1,2,7] If these drugs increase plasma renin activity, one would expect thirst to be a constant complaint, even when urine volume has been reduced. But the therapeutic effect in DI does not support this prediction. This facet has not yet been elaborated upon by BROWN et al.[7].

It seems, therefore, that although the renin-hypothesis of BROWN et al.[7] accounts for many phenomena related to the therapeutic effect of thiazides in DI, the experiments reported here as well as other data point to different mechanisms which may be involved in the mechanism of other drugs effective in DI, particularly anti-hyperglycemic drugs. E.g. chlorpropamide has been reported to have an effect similar to that of vasopressin[13]. It has been previously observed that vasopressin administration in vivo may reduce plasma renin activity in the dog[14,15]. The decreased plasma renin activity following chlorpropamide in our experiments in the rat may be related to the similarity of chlorpropamide action to that of vasopressin.

Résumé. La phénformine et la chlorpropamide, deux agents antihyperglycémiques, sont utilisés pour le traitement du diabète insipide. Ces deux drogues injectées à des rats diminuent le taux plasmatique de la rénine sans affecter la rénine du rein. La diminution de l'activité rénine plasmatique s'oppose à l'élévation que l'on trouve après injection de diurétiques du groupe chlorothiazide. On suppose que c'est cette élévation, qui produit l'effet antidiurétique de ces drogues dans le diabète insipide. L'activité de la rénine plasmatique ne peut donc expliquer l'effet antidiurétique de tous les composés utilisés dans ce diabète.

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Table I. Effect of phenformin on renin activity in the rat

	Control	Phenformin	p
Plasma renin activity	263 ± 30	116 ± 16	<0.001
Kidney renin activity	265 ± 33	203 ± 24	n.s.

Results expressed as mean ± S.E. In each group 9 rats. Plasma renin activity given as ng angiotensin liberated/ml plasma × 18 h. Kidney renin activity given as μg angiotensin liberated/g kidney × 1 h.

Table II. Effect of chlorpropamide on renin activity in the rat

	Control	Chlorpropamide	p
Plasma renin activity	308 ± 83	114 ± 19	<0.05
Kidney renin activity	357 ± 55	337 ± 47	n.s.

Results and units expressed as in Table I. In each group 9 rats.

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