

mone were absorbed. The complete absorption of all the testosterone in the pellets seems improbable for the melting point of the center was different from that of the periphery of the removed pellets. Furthermore, the weight loss of these pellets averaged but 0.05 mg (an average of 2.5 μ g per day), though the errors due to weighing and the absorption of substances from the body fluids makes an exact determination of the weight loss impossible.

The local or regional maintenance effected by the lower dosages indicates that testos-

terone acts directly on the testis and not by scrotal or general systemic stimulation.

Summary. Very small dosages of testosterone, administered intratesticularly to hypophysectomized rats, cause a regional maintenance of spermatogenesis. These dosages are much less than those necessary for complete maintenance of the sexual accessory glands. Testosterone has a direct action on the testis in the maintenance of spermatogenesis.

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Factors Influencing Ability to Counteract Gravitational Interference with Circulation, in the Rabbit.

ARTHUR LOCKE, R. B. LOCKE, AND A. P. McILROY.

From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh.

This is a report of a comparative study, in the rabbit, of a series of substances and procedures affecting ability to counteract the gravitational interferences with circulation imposed by the standing position. The substances studied were: paredrine, ephedrine, d-amphetamine, coramine, nicotinic acid amide, ascorbic acid, desiccated thyroid, sulfanilamide, sulfathiazole, and sodium nitrite. The procedures studied were: chilling, salt restriction, and bleeding from the heart and ear.

Method. New Zealand White male rabbits were used, between 3.0 and 4.5 kg in weight. The rabbits were chilled, in cool water,¹ until the rectal temperature had fallen to a point between 94 and 95°F. They were then dried and allowed to warm up again, spontaneously. When the rectal temperature had reached 96°F, they were placed in boxes forcing the rabbits to stand upright for one-half hour.

During this half hour of forced standing, temperature recovery proceeded without retardation in the rabbits able to counteract the

gravitational interferences with circulation imposed by the standing position. In rabbits with an impaired capacity for counteracting gravitational interference, the rate of temperature recovery slowed down during the period of forced standing and, in extreme instances, reached negative values (impending gravitational shock).¹

The extent of the temperature rise during the half hour of forced standing was measured: (1) in the absence of preliminary preparations other than were required to provide a true control for the test determination, and (2) one week later, during the period of action of the substance or procedure under study. Whenever practicable, an additional control determination was done one or 2 weeks after the test determination and averaged with the earlier control determination as a way of minimizing the magnitude of error due to spontaneous change.

An average test rise significantly greater than that observed during the control determinations was considered to be an indication of possible improvement as a result of the action of the substance or procedure tested. A significant decrease in rise was taken as an indication of converse impairment. A change

¹ Locke, A., Locke, R. B., McIlroy, A. P., and Chung, L., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **51**, 184.

TABLE I.
Effect of Ingested Sodium Nitrite on Susceptibility to Gravitational Shock in the Rabbit. Partial Counteraction by Paredrine and by d-Amphetamine. Absence of Counteraction by Coramine or Nicotinic Acid Amide.

Substance given	No. of rabbits	Avg temp. rise in degrees F during the half hour of forced standing		
		Control detn.	Test detn.	Change
A, alone	24	1.84†	0.88*	-0.96 ± .11‡
A plus B	10	.48*	1.44	+ .96 ± .16
A " C	10	.64*	1.47	+ .83 ± .16
A " D	10	.54*	.51	— .03 ± .10
A " E	5	.14*	— .08	— .22 ± .18

* Nitrite alone given. † No nitrite given. ‡ The probable error of the difference, as computed from Peter's formula.

A: Sodium nitrite, 60 mg/kg by mouth as a 20% solution in water. The nitrite was given approximately 20 min before subjection to the half hr of forced standing.

B: Paredrine hydrobromide, Smith, Kline and French, 2.5 to 7 mg by vein immediately prior to the interval of forced standing.

C: d-Amphetamine sulfate, Smith, Kline and French, 2.5 to 7 mg as described under B.

D: Coramine, Ciba, 75 mg as described under B.

E: Nicotinic acid amide, Breon, 10 mg as described under B.

All of the rabbits were subjected to a preparatory chilling on the day preceding test.

in rise, regardless of magnitude or sign, was not considered to be significant unless it was substantially more than 3 times as large as the modifying "probable error."

Sodium nitrite. Ingestion of this substance in sufficiently massive amount is known to produce effects in man directly attributable to a lessened capacity for counteracting gravitational interference with the blood supply to the heart and brain.² A parallel effect on the rabbit is indicated in Table I. Sodium nitrite affected 3 of the 24 rabbits in Group A so severely that a fall in body temperature was registered during the half hour of enforced standing. A corresponding proportion was so little affected that temperature recovery proceeded at a normal rate, despite the gravitational handicap of the erect position. The majority were affected to the intermediate extent described by the average. The differences were, in part, individual and not due altogether to chance or to differences in weight. Only those rabbits clearly impaired by the nitrite were used for the further tests for capacity for offsetting action (Groups B, C, D, and E). The control averages for these latter groups are, for this reason, necessarily lower than the test average for Group A alone, and non-identical.

² Kunkel, P., Stead, E. A., and Weiss, S., *J. Clin. Invest.*, 1939, **18**, 679.

Paredrine. Experimentation by Kunkel, Stead and Weiss² led to a recommendation of paredrinol as an ideal agent for offsetting nitrite effect. Paredrine was found by Nathanson and Engelberg³ to have a pressor action greater and more prolonged than that exerted by paredrinol. Paredrine produced a virtually complete counteraction of nitrite effect in the rabbit (Table I) and the parallel, partial counteraction of spontaneously heightened susceptibility to gravitational interference reported in Table IV.

Ephedrine. Ephedrine appeared to be equivalent to paredrine as an agent counteracting spontaneously heightened susceptibility to gravitational interference with circulation (Table IV).

d-Amphetamine. * The threshold dose of d-Amphetamine exerting central stimulation in the rat was found by Schulte and associates to be but one hundredth of that required by paredrine.⁴ d-Amphetamine appeared to be as effective as paredrine vs. nitrite, in the rabbit (Table I), but ineffective against spontaneous-

³ Nathanson, M. H., and Engelberg, H., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 239.

* This substance was tested at the suggestion of Dr. A. C. Ivy.

⁴ Schulte, J. W., Reif, E. C., Bacher, J. A., Lawrence, W. S., and Tainter, M. L., *J. Pharm. and Exp. Therap.*, 1941, **71**, 62.

TABLE II.
Effect of Blood Loss. Partial Counteraction by Ascorbic Acid.

Preparation	No. of rabbits	Avg temp. rise in degrees F during the half hour of forced standing		
		Control detn.	Test detn.	Change
F, alone	17	1.75*	0.55†	-1.20 ± .18
G, " "	26	1.99*	1.10†	- .89 ± .09
F plus H	7	— .24†	.93	+1.17 ± .25
G " " H	15	.81†	1.57	+ .76 ± .08

* No blood drawn. † Blood drawn, no ascorbic acid given.

F: 30 cc of blood drawn from the heart, 1 hr before the subjection to forced standing.

G: 30 cc of blood drawn from the marginal vein of the ear, 1 hr before the subjection to forced standing.

H: Ascorbic acid, Pfizer, 300 mg by vein immediately after solution in water and neutralization with sodium bicarbonate and immediately before the subjection to forced standing.

The control determinations for F, alone, and G, alone, were made 1 week before and after the test determinations. Those for F plus H and G plus H were made 2 weeks before and/or after. The rabbits were given a preparatory chilling on the day preceding test and weighed between 3 and 4.5 kg.

ly impaired capacity for counteracting gravitational interference with circulation (Table IV).

Coramine. Gunther, Engelberg and Strauss found that a lowered intramuscular pressure is not altered following paredrine but is consistently increased, together with the venous pressure, following coramine.⁵ Coramine possibly was more effective than paredrine vs. spontaneous impairment in ability to counteract gravitational effect, in the rabbit (Table IV), but appeared to be ineffective vs. nitrite (Table I)—an antithesis of the picture found for d-Amphetamine.

Nicotinic acid amide. This vitamin with a chemical structure similar to that of coramine was given in considerably smaller amount. In the dosage given, it was ineffective vs. nitrite (Table I) and of only doubtful effectiveness vs. spontaneously induced impairment (Table IV).

Blood loss. The bleedings from the heart (Table II) had a greater impairing effect than the bleedings from the ear but were more hazardous for the rabbit. A wide range of effect was noted. Only the rabbits clearly impaired were used for the further tests for counteraction by ascorbic acid.

Ascorbic acid. Ascorbic acid has been reported to prolong the survival time following

lethal injury⁶ and blood loss⁷ and to have effects on the water balance⁸ possibly pertinent to the findings reported in Table II and IV. For the rabbits there described, ascorbic acid was definitely effective vs. spontaneous impairment in capacity for counteracting gravitational interference and markedly effective vs. impairment as a result of blood loss.

Sodium chloride. Excessive reduction in the salt intake and excessive loss of sodium chloride in the sweat are known to produce an impaired tolerance for the erect position in man.⁹ Table III reports an equivalent, lessened capacity for counteracting gravitational interference with circulation in rabbits deprived of access to a salt lick, and a heightened capacity for such counteraction following restoration of access to salt.

Spontaneous impairment. None of the substances and procedures listed in Table IV appeared to produce more than a lessening of the pre-existing, spontaneous impairment present. None appeared able to confer on the rabbit a tolerance for the erect position in excess of that normally manifested in the absence of impairment. The more nearly the control rise approached a value of 2 or higher, the less frequently a heightened rise was ob-

⁵ Gunther, L., Engelberg, H., and Strauss, L., *Am. J. Med. Sc.*, 1942, **204**, 266, 271.

⁶ Ungar, G., *Nature*, 1942, **149**, 637.

⁷ Stewart, C. P., Learmouth, J. R., and Pollock, G. A., *Lancet*, 1941, **240**, 818.

⁸ Reid, M. R., *Physiol. Rev.*, 1943, **23**, 76.

⁹ Nadal, J. W., Pedersen, S., and Maddock, W. G., *J. Clin. Invest.*, 1941, **20**, 691.

TABLE III.
Effect of Change in Salt Intake.

Preparation	No. of rabbits	Avg temp. rise in degrees F during the half hour of forced standing		
		Control detn.	Test detn.	Change
Salt lick put in cage	12	1.53	2.08*	+0.55 ± .13
taken out	16	2.11	1.38†	— .73 ± .11
returned	14	1.01	1.66†	+ .65 ± .07

* 2 to 7 days after the placement of the salt rings in the cages and 6 days or longer after the last preceding determination.

† 6 to 14 days after the change in salt intake indicated.

The rabbits were given a preparatory chilling on the day before test and were given no salt other than that in the salt rings, excepting for the amounts naturally present in their food (hay and oats plus small amounts of bread and carrot).

TABLE IV.
Direct Effects of Paredrine, d-Amphetamine, Ephedrine, Coramine, Nicotinic Acid Amide, Ascorbic Acid, Desiccated Thyroid, and Chilling.

Substance or preparation given	Amt, mg	No. of tests	Avg temp. rise in degrees F during the half hour of forced standing		
			Control detn.	Test detn.	Change
Rabbits with a control rise of 0 to 1.3 degrees:					
None		18	0.88	1.05	+0.17 ± .07
B	2.5 to 15	14	1.00	1.41	+ .41 ± .10
C	.5 to 10	11	.74	.72	— .02 ± .13
J	2.5 to 5	13	.96	1.40	+ .44 ± .11
D	37 to 75	18	.84	1.53	+ .69 ± .13
E,E'	10 to 20	27	.87	1.11	+ .24 ± .09
H'	300	11	.66	1.27	+ .61 ± .12
L		16	.93	1.54	+ .61 ± .09
M'		13	.89	1.92	+1.03 ± .10
M''		20	.84	1.64	+ .80 ± .11
Rabbits with a control rise of more than 1.3 degrees:					
None		11	1.85	1.88	+ .03 ± .07
B	2.5 to 15	20	2.04	1.93	— .11 ± .13
C	.5 to 5	20	1.84	1.46	— .38 ± .10
D	75	14	1.71	2.06	+ .35 ± .12
E,E'	10 to 20	16	1.81	1.92	+ .11 ± .07
H'	300	18	1.86	2.02	+ .16 ± .08
L		9	1.81	1.94	+ .13 ± .04
M'		8	2.06	2.25	+ .19 ± .07
M''		22	1.92	2.31	+ .39 ± .11

B, C, D, E: See Table I.

E': Nicotinic Acid, Eastman, as described under B.

H': Ascorbic Acid, Pfizer, by mouth, 1½ hours before the subjection to forced standing.

J: Ephedrine sulfate, Mallinckrodt, as described under B.

L: Subjection to preparatory chilling on the preceding day. No chilling had been given for one week or longer preceding the control determinations in this group. A chilling on the preceding day, following one week without chilling, was given for all of the determinations in H', since ascorbic acid has appeared to be ineffective in the absence of such preparation.¹ Whether or not a preceding chilling had been given appeared to be less material for the other agents in this table. Each test determination was, excepting for group L, done exactly as the controls (that is, if a preparatory chilling was given on the day before the control determination, an equivalent chilling was given, also, on the day before the test determination).

M': Desiccated Thyroid, Parke-Davis, 200 to 300 mg by mouth daily for 8 days. The thyroid feedings at this level produced an average weight loss of 0.3 kg and death within 5 to 8 days in the proportion of 5/18.

M'': Desiccated Thyroid, 100 mg by mouth daily for 7 to 13 days. Supplementary feedings of ascorbic acid and of vitamin B complex were given to a few of the rabbits in this group, without observed material effect on outcome. The feedings at this level were better tolerated, producing no deaths and an average total weight loss of only 0.09 kg.

TABLE V.
Effects of Sulfanilamide and Sulfathiazole.

Substance given	Amt, g/kg	Avg blood level, total mg %	No. of rabbits	Avg temperature rise, F, during the half hour of forced standing		
				Control detn.	Test detn.	Change
SA	0.11 to .19	7.7	12	1.48	1.14	-0.34 ± .07
	.20 to .29	14.1	7	1.99	1.44	- .55 ± .24
ST	.11 to .23	6.0	7	2.20	2.21	+ .01 ± .08
	.32 to .48	8.8	5	1.96	1.66	- .30 ± .12

SA: Sulfanilamide, Winthrop, by mouth 3 hours before the subjection to forced standing.
ST: Sulfathiazole, Maltbie, by mouth as described for SA.

served after administration of the substance or procedure under test.

Hardening. Hardening has been suggested as a corrective measure vs. the impaired capacity for counteracting gravitational interference associated with effort syndrome.¹⁰ Both exercise and chill appear to contribute to the hardening process. One preliminary chilling to 94°F on the day preceding test produces a lessening of impairment so large, in rabbits unsubjected to chilling for a week or longer preceding test,¹ as to require the rigorous control for this factor maintained throughout the tests made for this report.

Desiccated thyroid. The effectiveness of this principle vs. spontaneous impairment in ability to counteract gravitational interference with circulation, in the rabbit, already has been indicated in reference 1.

Sulfanilamide, sulfathiazole. The rabbits tested for effect by these drugs were not harboring infection to an extent producing observed effect on weight or temperature—a qualification of importance because infection lessens the tolerance for the upright position¹ and sulfa drugs are not ordinarily given in

the absence of infection. Virtually no impairment was apparent from the amounts of sulfathiazole given, during the time covered by the tests. Moderate impairment was observed with the sulfanilamide.

Summary. The following substances and procedures have been observed to have effects on the rate of temperature rise in chilled rabbits confined to an erect position (1) indicative of tendency to produce impairment in ability to counteract gravitational interference with circulation: blood loss, decreased salt intake, "softening" inaction, infection, sodium nitrite, and, to a considerably less marked extent, sulfanilamide; and (2) indicative of tendency to produce a converse lessening of pre-existing impairment: paredrine, ephedrine, coramine, ascorbic acid, thyroid, and hardening. Paredrine had a parallel effect vs. nitrite-induced impairment. Coramine, effective vs. spontaneous impairment, appeared to be ineffective vs. nitrite-induced impairment. d-Amphetamine, in direct contrast, gave indications of effectiveness vs. nitrite-induced impairment but not vs. spontaneous impairment. Impairment as a result of blood loss was substantially lessened following intravenous sodium ascorbate, in massive dosage.

¹⁰ Lewis, T., *The Soldier's Heart and the Effort Syndrome*, 1919.