TABLE I.

Time for 90% reduction of methylene blue (1:50,000) by a phosphate buffer suspension of luminous bacteria (*Achromobacter Fischeri*), with and without added substrate.

Suspension	Substrate	Reduction time		
		Deaerated by evacuation	Deaerated by hydrogen	Deaerated by nitrogen
Unwashed	—	27′	7′	7′ 15″
Washed 4 times	—	>3 days	>6 hr.	>6 hr.
,, ,, ,,	M/10 glucose	27′	8′	8′
,, ,, ,,	M/10 alpha methyl glucoside	>3 days*	29′	29′

*Barely perceptible decolorization in 6 hrs, that amounted to approximately 30% reduction over a period of 3 days.

It is a matter of considerable importance that the usual technique of evacuation gave only a vague evidence for the dehydrogenation of alpha methylglucoside. This substance has been found to inhibit aerobic respiration of washed cells[15] and would thus lengthen the time for removal of oxygen. Yet the new method shows that it is readily dehydrogenated. In addition to use with bacterial suspensions, this method should work equally well with any tissue or tissue preparation which can be delivered with a pipette.

9242

Effect of Testosterone on Somatic Growth.

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It has been shown by Steinach and Holzknecht[1] that ovarian hormones inhibit growth. They found that implantation of ovaries into male guinea pigs or rats decreased their growth rate in every case if the transplant took. Later, these findings were confirmed by Bugbee and Simond,[2] and Spencer, *et al.*,[3, 4] using oestrin preparations. Spencer, *et al.*,[5] came to the conclusion that the growth in-

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5 Spencer, J., D'Amour, F. E., and Gustavson, R. G., *Endocrinol.*, 1932, **16**, 647.

hibition is due to a depression of pituitary function by oestrin. Retardation of growth in the rat under the influence of oestrin administration has also been reported by Korenchevsky and Dennison[6] and Halpern and D'Amour.[7] While the growth hormone production of the pituitary is thus interfered with, the actual size of the hypophysis increases so much that tumor formation may occur (Zondek,[8] Cramer and Horning,[9] McEuen, *et al.*[10] The inhibition of pituitary function is also evidenced by the atrophy of the gonad which ensues under the influence of oestrin administration. This has been shown by Herrmann and Stein[11] who, with oestrogenic placental and corpus luteum extracts, obtained testicular atrophy in the rat and other mammals. In the female rat, gonad atrophy was observed following the injection of oestrogenic ovarian extracts or follicular fluid by Terada.[12] Moore and Price,[13] who made similar observations with purified ovarian hormone preparations, advanced the theory that injections of gonadal hormones inhibits the production of gonad-stimulating hormone by the hypophysis. The fact that oestrin may cause gonad atrophy was later confirmed repeatedly (Wade and Doisy,[14] Ihrke and D'Amour,[15] Leonard, *et al.*,[16] Spencer, *et al.*[5]).

In connection with these experiments it seemed of interest to establish whether testosterone would have similar effects. In order to obtain evidence concerning this question we treated rats chronically with large doses of testosterone.* The first series consisted of 12 normal and 9 castrate males, and 12 normal and 11 castrate females. It was divided into two groups as shown in Table I. The first group received daily subcutaneous injections of 200γ of testosterone in corn oil, beginning at the 25th day of life. Their controls, the second group, received a similar dose of cholesterol in oil or oil alone. In the normal males treated with testosterone the testes and scrota were obviously much smaller than in those not

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8 Zondek, B., *Lancet*, Jan. 4, 1936, p. 10.

9 Cramer, W., and Horning, E. S., *Lancet*, May 9, 1936, p. 1056.

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12 Terada, M., *Japan. med. World*, 1927, **7**, 233.

13 Moore, C. R., and Price, D., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 1.

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* The authors are indebted to Dr. Erwin Schwenk of the Schering Corporation for the testosterone used in these experiments.

TABLE I.

	Duration of treatment days	Body weight increase in gm. Testosterone No. of rats	Extreme individual variations	Aver.	Controls (oil) No. of rats	Extreme individual variations	Aver.
Normal males	432	6	334-374	353	6	273-394	334
Castrate* "		5	301-437	358	4	330-475	380
					Controls (cholesterol)		
Normal females	272	6	164-260	220	6	139-201	169
Castrate "		5	184-260	230	6	173-253	204

*Injections were omitted during 54 days owing to lack of supply of Testosterone.

TABLE II.

Males	No. of rats	Body weight increase in gm. Extreme individual variations	Aver.	Aver. endocrine weights Testes	Sem. Vesicles and Prostate	Epidid.	Adrenals	Pituitary
Testosterone	5	60-100	75.4	1.324	1.557	.415	.023	.007
Cholesterol	5	59- 89	77.6	2.133	.365	.327	.027	.006
Untreated	5	59- 97	80.2	2.429	.830	.419	.024	.007
Females				Ovaries				
Testosterone	5	53- 77	63.8	.016			.022	.007
Cholesterol	5	34- 53	48.6	.031			.034	.006
Untreated	5	34- 51	44.2	.037			.032	.006

treated with this hormone. The normal females treated with testosterone went into permanent vaginal dioestrus within the first 2 months of treatment, while the cycles in the cholesterol treated controls remained normal. The organ weights of this group are not recorded in the table as the animals have been kept alive for further observation.

In a second series, consisting of 15 normal males and 15 normal females, 5 animals of each sex received the high dose of 2 mg. of testosterone in corn oil daily by subcutaneous injection. Five other males and 5 females received the same dose of cholesterol in oil, while the remaining 5 of each sex were left untreated. The animals were 36 to 38 days of age at the initiation of treatment and they were sacrificed on the 23rd day of the experiment. Their somatic weights and the weights of their endocrines are summarized in Table II. In all testosterone treated animals the testes and ovaries showed atrophy and the females became dioestric within the first 7 days of testosterone treatment, while no such change was observed in any of the control groups. Histologically the mammary glands of all the testosterone treated animals were well developed and showed some secretion, while no development or secretion was observed in untreated or cholesterol treated females and only slight development was seen in the untreated males. The fact that the mammary gland of the normal rat (not that of the castrate) shows some development and that testosterone will stimulate the development of the mammary gland, both in the male and in the female castrate, has been described in previous communications.[17, 18] As is seen in Tables I and II there is no sign of somatic growth inhibition in either group under the influence of testosterone; indeed, there appears to be a suggestion of growth stimulation, at least in the normal female. The hypophyses likewise failed to show any stimulation comparable with that obtained by us[19] with oestrin under similar conditions.

Summary. No somatic growth inhibition was observed in the rat even when treated with very large doses of testosterone, although these same doses proved sufficient to inhibit gonad development in both sexes. It has been found that doses of testosterone which suffice to inhibit the growth of the gonad in both sexes and to cause

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[18] McEuen, C. S., Selye, H., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 56.

[19] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1377.

permanent vaginal dioestrus in the female do not cause hypertrophy of the hypophysis in either sex. In these respects, the effect of testosterone on somatic and hypophyseal growth differs from that of oestrone.

9243

Action of Arasaponins A and B.

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The Chinese drug San-ch'i has been botanically identified as *Gynura pinnatifida*,[1, 2] although it has been named *Aralia bipinnatifida*[3] by Y. H. Chao. The plant is a short herb grown in southwestern China. The root is the part that has been advocated in Chinese medicine chiefly as an astringent and hemostatic. In a previous note,[3] one of us (T. Q. C.) reported the isolation of 2 saponins from San-ch'i, arasaponin A and arasaponin B. The former melts at 195-210°C., has a specific rotation $[\alpha]_D$ +23°, and conforms to the empirical formula $C_{30}H_{52}O_{10}$; while arasaponin B melts at 190-200°C., is also dextro-rotatory ($[\alpha]_D$ +8°), and has an empirical formula $C_{23}H_{38}O_{10}$. Both substances are moderately soluble in water, foam forming upon agitation.

Hemolysis experiments, 14 in all, were carried out according to the method described by Ponder[4] with both saponins at 37°C. It was found that arasaponin A laked a guinea pig's red cells in the concentration of 1:4000 within 2 hours 43 minutes. Solutions of 1:2500, 1:2000, 1:1000, 1:750, 1:500, and 1:250 were more readily effective, but it still required 1 hour 14 minutes. Weaker concentrations, such as 1:5000, 1:8000, and 1:10,000, had no hemolytic action at the end of 8 hours. Arasaponin A also hemolyzed dogs' and monkeys' blood, but the latent period was very long. For example, a 1:250 solution laked a dog's red cells in 4 hours 34

1 *Botanica Nomenclature*, Commercial Press, Shanghai, 1917, p. 23.

2 Chen, C. J., *Encyclopedia of Chinese Materia Medica*, The World Press, Shanghai, 1924, **1**, 38.

3 Chou, T. Q., and Chu, J. H., *Proc. Chinese Physiol. Soc.*, Tsingtao Meeting, 1936, p. 12.

4 Ponder, E., *The Mammalian Red Cell and the Properties of Hæmolytic Systems*, G. Borntraeger, Berlin, 1934, p. 139.