TABLE I. Enhancement of Myotrophic and Androgenic Responses by Aqueous Suspension of Steroids.

Treatment		Guinea pigs	Dose,	Body wt		Increase		
				Initial,	Increase,	Temporal muscles (M)	Sem. ves. + pros. (A)	
Steroid	Vehicle	No.	mg/day	g	g	mg*	mg*	M/A†
Control		14		539	54	(690)	(550)	
Testosterone	Oil	4	.25	535	56	83	129	
	Susp.	4	.25	564	86	184	1072	.17
Androstanediol-3 α, 17 α	"	5	.25	551	79	256	960	.27
Testosterone	Oil	4	.50	539	85	131	114	1.15
	Susp.	4	.50	584	79	426	1756	.24
Test. propionate	Oil	4	.50	574	77	456	2230	.21
Androstanediol-3 α, 17 α	Susp.	4	.50	573	85	452	1560	.29

* The values are the increases above the control values which are in parentheses.
† M/A = The growth of the temporal muscles divided by the growth of the seminal vesicles and prostates.

plus its smaller effect on the accessory sex organs in agreement with pellet studies(7) now makes it possible to carry out clinical studies with this interesting steroid. The extremely low solubility of androstanediol-3 α, 17 α in oil(9) has previously made such studies difficult.

Summary. An aqueous microcrystalline suspension of testosterone was 3 times more effective in stimulating the growth of the temporal muscles and 9-16 times more effective on the accessory sex organs than an olive oil solution of testosterone and as effective as a sesame oil solution of testosterone propionate. An aqueous microcrystalline suspension of androstanediol-3 α, 17 α was slightly more effective on the temporal muscle and less effective on the accessory sex organs than a similar suspension of testosterone.

9. Kochakian, C. D., *Am. J. Physiol.*, 1950, v160, 53.

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Protein Anabolic Activity of Pregnant Mares' Urine.* (18259)

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The presence of a protein anabolic factor(s) in androgenic male urine extracts was reported(1) 15 years ago. A similar factor(s) now has been found in pregnant mares' urine.

Procedure. Pregnant mares' urine was extracted† by the method used for human male urine(1) with minor modifications. The neutral extracts were fractionated‡ by separation into ketonic and non-ketonic portions by means of Girard's T reagent and were then further fractionated by chromatography with magnesium silicate as adsorbent(2). The

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1. Kochakian, C. D., and Murlin, J. R., *J. Nutrition*, 1935, v10, 437.

† The neutral extracts were prepared by the Ayerst, McKenna and Harrison Ltd.

‡ The following phase of the program was carried out at Rochester with the assistance of Betty Beall.

TABLE I. Characteristics and Solubility in Olive Oil of Protein Anabolic Fractions of Pregnant Mares' Urine.

Fraction	Eluant	Wt, g	Physical characteristics	Solubility in olive oil
	Total ketonic.			
LH-62-1	(α-ketonic)	0.95	Fluid, dark red oil	Slight ppt.
	Ketonic fractions.			
C-3-17-I	Benzene to benzene: ether (97, 95, 90, 75)*	4.94	Taffy-like	No ppt.
II	Benzene: ether (75, 50) Ether, ether: methyl alcohol (90)	1.55	Brittle solid	Slight ppt.
III	Ether: methyl alcohol (90, 50) Methyl alcohol	2.27	Powder	Copious dark red ppt.

* The figures in the parentheses indicate the percent of the first solvent in the respective mixtures.

effectiveness of these procedures was determined by the Zimmermann(3), Pincus(4) and 2,4 dinitrophenylhydrazine color tests and androgenic(5), estrogenic (6) and protein anabolic(7) assays in the rat and the myotrophic-androgenic test in the guinea pig(8). Since the results indicated the presence of the protein anabolic factor, larger amounts were prepared and the activity was finally established in the dog. The 2 ketonic fractions studied in the dog were prepared as follows§. A total ketonic fraction, 15 g, was chromatographed to give the following 4 eluates: with carbon tetrachloride 1.753 g, benzene 1.640 g, benzene-methyl alcohol (95:5) 10.946 g, and methyl alcohol 0.401 g. The third and largest fraction was rechromatographed and the eluates pooled into 4 fractions (Table I). In addition an α-ketonic fraction (Table I) was prepared from another large neutral extract¶. The fractions were dissolved in acetone and sufficient olive oil was added to give a concentration of 100 mg/ml but some of the fractions contained material which was insoluble in the oil (Table I).

The metabolism studies‖ were carried out essentially as described earlier(1,9,10). Dog 68 was a dark brown, short-haired mongrel probably of terrier and hound lineage. Dog 72 was a white short-haired mongrel of predominantly bull and terrier lineage. The dogs were castrated and maintained in individual metal metabolism cages. They were fed a diet consisting of beef heart 52, cracker meal 22, hydrogenated vegetable oil 5.2, cod liver oil 1.3, dry brewers' yeast (Fleischmann's No. 2019) 1.3, cellu flour 1.3, and Wesson's salt mixture 1.3. The amount fed was estimated by the formula of Cowgill and Drabkin(11) but small changes had to be made to establish and maintain constant body weight and nitrogen equilibrium. The urine collections were made under toluene and the dogs were catherized at 2 day intervals, occasionally one or 3 day intervals. The first

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3. Holtorff, A. G., and Koch, F. C., *J. Biol. Chem.*, 1940, v135, 377.

4. Pincus, G., *Endocrinology*, 1943, v32, 176.

5. Kochakian, C. D., *Endocrinology*, 1938, v22, 181.

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7. Kochakian, C. D., *Am. J. Physiol.*, 1950, v160, 53.

8. Kochakian, C. D., Humm, J. H. and Bartlett, M. N., *Am. J. Physiol.*, 1948, v155, 242.

§ These preparations were made by Ayerst, McKenna and Harrison Ltd. in accordance with the results of the extensive exploratory studies at Rochester.

¶ This fraction was prepared by Dr. K. Dobriner of the Sloan-Kettering Institute.

‖ William van de Mark and Arthur Sakamoto assisted in these studies.

9. Kochakian, C. D., and Murlin, J. R., *Am. J. Physiol.*, 1936, v117, 642.

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11. Cowgill, G. R., and Drabkin, D. L., *Am. J. Physiol.*, 1927, v81, 36.

TABLE II. Protein Anabolic Factor(s) of Pregnant Mares' Urine.

Treatment*	Dose mg/day	Dose days	Max. N retained, g/day	Incr. body wt, kg
	Dog 68†			
Testosterone§	20	13	1.32	.45
	100	2		
α-ketonic, LH 62-1	150	5	1.40	.45
	Dog 72‡			
Testosterone§	20	13	1.03	.36
	100	5		
Ketonic, C-3-17-II	200	5	0.62	.36
Ketonic, C-3-17-III	200	8	0.90	.45

* First exp. 120 days after castration.
† Body wt, 9.3 kg; N intake, 5.40 g/day.
‡ Body wt, 11.2 kg; N intake, 6.60 g/day.
§ Aqueous micro-crystalline suspension at 100 mg/ml.

experiment was not begun until 120 days after castration. The dietary nitrogen was determined by the macro-Kjeldahl procedure with distillation of aliquots by the micro procedure. The urine nitrogen was determined by the micro-Kjeldahl method.

Results. The testosterone and the urine fractions (Table II) gave the typical nitrogen retention and body weight responses (1, 9,10). The urinary nitrogen excretion promptly decreased to a minimum where it was maintained until cessation of injections when an extra excretion of nitrogen occurred. The body weight increased with the retention of nitrogen and decreased slightly on cessation of injections. Table II shows the maximum nitrogen retained (average of several periods) and the maximum increase in body weight.

Testosterone at 20 mg per day in a micro-crystalline suspension produced a very marked drop in urinary nitrogen and an increase in body weight. The smaller dog interestingly responded to a greater degree than the slightly larger dog. The total ketonic fraction at 5 to 7.5 times the dose of testosterone produced effects identical with those of the testosterone as did the 2 ketonic fractions at 10 times the dose of testosterone. The first ketonic fraction (C-3-17-1) showed no protein anabolic properties in the rat but this fraction was not studied in the dog.

Discussion. The establishment of protein anabolic activity in the ketonic fraction of extracts of pregnant mares' urine does not exclude the presence of similar properties in other fractions. Furthermore, the activity in the ketonic materials may be due to more than one compound since the 2 chromatographic fractions exhibited a high degree of activity. Most of the activity probably is in the α-steroid(s). Many ketonic and also non-ketonic steroids now are known to stimulate growth(7,8,12,13).

The relative potency of these extracts cannot be assessed until the minimum effective dosages are determined. Furthermore, the testosterone was not studied in oil solution. The micro-crystalline suspension was arbitrarily chosen in order to compare it later with an oil solution. A rough quantitative comparison can be made between the urine extracts and the testosterone. The micro-crystalline suspension is 3 times as effective in the myotrophic and 9-16 times as effective in the androgenic test in the guinea pig as an oil solution of testosterone(14). On this basis, the ketonic fractions may be considered to possess at least one half the potency of testosterone. Furthermore, the maximal responses were identical or very similar to those produced by testosterone. Therefore, the active compounds must be considered of relatively high potency.

Summary. Pregnant mares' urine has been demonstrated to contain a relatively high protein anabolic activity as determined by nitrogen balance and body weight studies in castrated dogs. A large proportion of the activity is in the α-ketonic fraction of a neutral fat-soluble extract and is probably due to more than one compound.

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