CCV. THE ASSAY OF CRYSTALLINE MALE SEXUAL HORMONE (ANDROSTERONE).

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From the Lister Institute.

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RUZICKA *et al.* [1934] have prepared from *epidihydrocholesterol*, an oxyketone having the formula $C_{19}H_{30}O_2$, which in composition, melting point, optical activity and effect in stimulating comb-growth in capons appears to be identical with the crystalline hormone isolated from human urine by Butenandt and his co-workers [1932; 1934]. Butenandt [1934] found that one capon unit was contained in about 150–200 γ of his preparation of androsterone, whilst Tschopp [Ruzicka *et al.*, 1934, p. 1398; 1935, p. 211], using an assay technique similar to that used by Butenandt, found the same value for Ruzicka's androsterone. Tschopp [Ruzicka *et al.*, 1934, p. 1401], using only a small number of castrated rats, has also found that the prostate, seminal vesicles and penis became enlarged after the injection of Ruzicka's androsterone.

This paper contains the results of our assay of androsterone synthesised by Ruzicka using the method and definition of the rat unit described in a previous paper [Korenchevsky and Dennison, 1934, 2, p. 1498]. The preliminary results with this material were published elsewhere [Korenchevsky, 1935].

In addition to the effects obtained on the secondary sexual organs, the changes in some of the other organs investigated are also described.

Technique.

The experiments were performed on 74 castrated rats belonging to 15 litters. The technique used was described in detail in our previous papers [Korenchevsky *et al.*, 1932; 1933; 1934]. Since rats are more liable to develop pneumonia during the winter than during the summer months, for about 10 days before the experiments the doses of cod-liver oil and raw liver were greatly increased with the object of increasing their resistance to infection. The effect on the deposition of fat was very irregular, the amount of fat in different rats varying greatly. The results given by the calculation of the weight of the organs per unit of body weight were therefore a little less regular than the actual weights.

1.2 g. of androsterone were received from Prof. Ruzicka in three batches, which were carefully mixed and kept in a vacuum in the cold store. A weighed amount of androsterone was dissolved in alcohol and poured into warm olive oil. Traces of alcohol were then removed as completely as possible *in vacuo* at about 80–85°. We stress this point of complete removal of alcohol, since any alcohol left in the oily solution may alter the absorption of androsterone from the oil and may therefore change the results of assay.

The oily solution of androsterone was filled into ampoules and sterilised in the steamer for 30 minutes on 3 consecutive days. 0.2 ml. per day of oily solution of different strengths was injected subcutaneously, 0.1 ml. being injected twice a day for 7 consecutive days and the rats killed on the 8th day.

Doses of 67, 200, 450, 600, 900, 1350 and 1800 γ per day were used. Since the effect produced by 67 γ was so small as to be within the physiological variations and only 2 rats were injected with this dose, the effect of this dose is not included in the tables or curves.

All the rats were castrated before sexual maturity and the experiments started from 28 to 42 days after castration, the final age varying from 53 to 66 days. One or two rats in each litter were used for the control group, the remaining litter-mates being injected. The groups of injected litter-mates contained from 1 to 4 rats according to the number of rats in the litter.

In Table I are given the number of rats in the litter, the age and the average final weights of the rats in each group in each litter.

Table I.

Number of rats in each litter. Age and average final weights of rats in each group of each litter.

No. of litter	No. of rats in litter	Age at castra- tion (days)	No. of days after injec- tion started	Weights of rats (g.)							
				Control rats	Rats injected with (γ)						
					67	200	450	600	900	1350	1800
1	4	23	42	195	220	193	—	192	—	—	—
2	4	22	42	207	—	248	—	188	—	—	—
3	4	21	40	209	247	244	—	200	—	—	—
4	4	28	38	209	—	—	207	—	228	—	—
5	4	28	38	230	—	—	—	—	231	—	226
6	4	21	36	225	—	—	218	—	254	—	—
7	5	26	30	210	—	—	179	—	—	—	201
8	5	25	30	164	—	—	—	—	187	—	199
9	5	27	32	215	—	—	—	—	—	202	—
10	5	28	37	231	—	224	223	—	—	—	—
11	6	26	37	209	—	222	—	219	—	—	—
12	7	25	37	208	—	—	207	194	207	—	—
13	7	20	37	168	—	—	—	198	190	201	—
14	5	26	28	214	—	—	—	—	—	232	—
15	5	21	32	177	—	—	—	—	—	—	165

The effect on the prostate, the seminal vesicles and the prostate and seminal vesicles weighed together.

The results obtained are summarised in Tables II–VI and are represented graphically by the curves in Figs. 1–4. The latter give the relation between the dose of androsterone and the percentage increase obtained in the weight of the organ.

Since the seminal vesicles weigh only one-fifth or less of the weight of the prostate and since the percentage increase in weight under the influence of different doses of androsterone (Table VI) is roughly parallel with that of the prostate, the curves representing the changes in the weight of the prostate alone and of the prostate with seminal vesicles were very similar (Figs. 1–4). For economy of space we do not give the curves for the seminal vesicles since these data are unsuitable for the purpose of assay. The changes obtained in the weights of the seminal vesicles (see average figures, Table VI), besides being more irregular, do not represent such a simple proportional relationship to the dose as those of the prostate and of the prostate weighed with the seminal vesicles.

Table II. *Influence on prostate.*

The actual average weights of the prostate of rats in each group of each litter and the percentage increase after the injection of androsterone.

No. of litter	Control rats	Rats injected with (γ)					
		200	450	600	900	1350	1800
		Weights (mg.).					
1	51	68	—	118	—	—	—
2	52	80	—	130	—	—	—
3	52	78	—	105	—	—	—
4	53	—	131	—	180	—	—
5	56	—	—	—	190	—	194
6	58	—	106	—	164	—	—
7	59	—	110	—	—	—	207
8	52	—	—	—	152	—	172
9	52	—	—	—	—	161	—
10	59	92	131	—	—	—	—
11	58	86	—	147	—	—	—
12	52	—	111	156	170	—	—
13	46	—	—	101	188	197	—
14	54	—	—	—	—	213	—
15	51	—	—	—	—	—	181
Average	54	81	118	126	174	190	189
Percentage increase in each individual group.							
1	—	33	—	131	—	—	—
2	—	54	—	150	—	—	—
3	—	50	—	102	—	—	—
4	—	—	147	—	239	—	—
5	—	—	—	—	239	—	246
6	—	—	83	—	189	—	—
7	—	—	86	—	—	—	251
8	—	—	—	—	192	—	231
9	—	—	—	—	—	210	—
10	—	56	122	—	—	—	—
11	—	48	—	150	—	—	—
12	—	—	113	200	227	—	—
13	—	—	—	120	308	328	—
14	—	—	—	—	—	294	—
15	—	—	—	—	—	—	255
Average	—	48	110	142	232	277	246

Fig. 1.

Fig. 2.

Fig. 1. Relation between dose of androsterone and percentage increase in actual weight of prostate.

Fig. 2. Relation between dose of androsterone and percentage increase in weight of prostate calculated per unit of body weight.

Table III. *Influence on prostate.*

The average weights of the prostate calculated per 200 g. of body weight in each group of each litter and the percentage increase after the injection of androsterone.

No. of litter	Control rats	Rats injected with (γ)					
		200	450	600	900	1350	1800
		Weights (mg.).					
1	52	70	—	123	—	—	—
2	50	65	—	140	—	—	—
3	50	64	—	105	—	—	—
4	51	—	127	—	159	—	—
5	49	—	—	—	164	—	171
6	52	—	97	—	129	—	—
7	56	—	124	—	—	—	213
8	63	—	—	—	163	—	173
9	48	—	—	—	—	162	—
10	51	82	120	—	—	—	—
11	56	78	—	136	—	—	—
12	51	—	108	161	164	—	—
13	56	—	—	102	198	196	—
14	50	—	—	—	—	185	—
15	58	—	—	—	—	—	219
Average	53	72	115	128	163	181	194

Percentage increase in each individual group.

1	—	35	—	136	—	—	—
2	—	30	—	180	—	—	—
3	—	28	—	110	—	—	—
4	—	—	149	—	212	—	—
5	—	—	—	—	235	—	249
6	—	—	87	—	148	—	—
7	—	—	121	—	—	—	280
8	—	—	—	—	159	—	175
9	—	—	—	—	—	238	—
10	—	61	135	—	—	—	—
11	—	39	—	143	—	—	—
12	—	—	112	216	221	—	—
13	—	—	—	82	254	250	—
14	—	—	—	—	—	270	—
15	—	—	—	—	—	—	278
Average	—	39	121	145	205	253	246

Fig. 3.

Fig. 4.

Fig. 3. Relation between dose of androsterone and percentage increase in actual weight of prostate with seminal vesicles.

Fig. 4. Relation between dose of androsterone and percentage increase in weight of prostate with seminal vesicles calculated per unit of body weight.

Table IV. *Influence on prostate with seminal vesicles.*

The actual average weight of the seminal vesicles with prostates of rats in each group in each litter and the percentage increase after the injection of androsterone.

No. of litter	Control rats	Rats injected with (γ)					
		200	450	600	900	1350	1800
Weights (mg.).							
1	62	83	—	143	—	—	—
2	65	97	—	174	—	—	—
3	58	88	—	128	—	—	—
4	67	—	158	—	211	—	—
5	69	—	—	—	221	—	233
6	69	—	135	—	204	—	—
7	70	—	135	—	—	—	263
8	64	—	—	—	181	—	215
9	67	—	—	—	—	202	—
10	72	110	155	—	—	—	—
11	68	98	—	171	—	—	—
12	63	—	132	181	201	—	—
13	55	—	—	125	216	228	—
14	63	—	—	—	—	252	—
15	61	—	—	—	—	—	217
Average	65	95	143	154	206	224	232
Percentage increase in each individual group.							
1	—	34	—	131	—	—	—
2	—	49	—	152	—	—	—
3	—	52	—	121	—	—	—
4	—	—	136	—	215	—	—
5	—	—	—	—	220	—	238
6	—	—	96	—	196	—	—
7	—	—	93	—	—	—	276
8	—	—	—	—	183	—	236
9	—	—	—	—	—	201	—
10	—	53	115	—	—	—	—
11	—	44	—	151	—	—	—
12	—	—	109	187	219	—	—
13	—	—	—	127	292	315	—
14	—	—	—	—	—	300	—
15	—	—	—	—	—	—	256
Average	—	46	110	145	221	272	252

From the comparison of Fig. 1 with Fig. 2 and of Fig. 3 with Fig. 4 it is clear that the changes calculated from the actual weights of the organs and from those per 200 g. of body weight are similar.

We also found this to be so in our previous investigation on the effect of a testicular hormone preparation extracted from human urine [Korenchevsky *et al.*, 1932; 1933, 1, 2]. However, from the comparison of the data given in the papers just cited with those in Tables IV and V it follows that the variation in the percentage increase obtained in different litters with the same dose was less with urine preparations than that observed in the present experiments with androsterone.

In spite of this difference between the individual litters, in both cases (with testicular hormones extracted from human urine and with androsterone) the relation between the dose and the average percentage increase in the weight of the prostate or prostate with seminal vesicles appeared to be directly proportional, within a certain range of the doses. In the case of androsterone this part of the curve lies between the doses 200 and 900 γ and can for practical purposes be represented by a straight line (the continuous line in Figs. 1-4). When the

Table V. *Influence on prostate with seminal vesicles.*

The average weights of the prostate with seminal vesicles calculated per 200 g. of body weight and the percentage increase after the injection of androsterone.

No. of litter	Control rats	Rats injected with (γ)					
		200	450	600	900	1350	1800
		Weights (mg.).					
1	64	86	—	149	—	—	—
2	63	78	—	176	—	—	—
3	56	72	—	128	—	—	—
4	64	—	153	—	187	—	—
5	60	—	—	—	191	—	206
6	61	—	124	—	161	—	—
7	66	—	152	—	—	—	271
8	78	—	—	—	194	—	217
9	62	—	—	—	—	202	—
10	62	98	142	—	—	—	—
11	66	89	—	159	—	—	—
12	61	—	129	187	194	—	—
13	67	—	—	126	227	227	—
14	59	—	—	—	—	218	—
15	69	—	—	—	—	—	263
Average	64	85	140	154	192	216	239
Percentage increase in each individual group.							
1	—	34	—	133	—	—	—
2	—	24	—	179	—	—	—
3	—	29	—	129	—	—	—
4	—	—	139	—	192	—	—
5	—	—	—	—	218	—	243
6	—	—	103	—	164	—	—
7	—	—	130	—	—	—	311
8	—	—	—	—	149	—	178
9	—	—	—	—	—	226	—
10	—	58	129	—	—	—	—
11	—	35	—	141	—	—	—
12	—	—	111	207	218	—	—
13	—	—	—	88	239	239	—
14	—	—	—	—	—	269	—
15	—	—	—	—	—	—	281
Average	—	36	122	146	197	245	253

whole range of the doses of androsterone used was included in the curve, a cubic curve was found to fit the results most closely (dotted lines in Figs. 1-4). As can be seen from the curves, there is little difference between the cubic curve and a straight line with doses of 200-900 γ .

The next doses used (1350 and 1800 γ) lie on a flattened portion of the curve. As we used fewer litters for this part of the curve (as not being suitable for assay), too much reliability must not be attached to the slight dip in the end of the curve, which is shown by the highest dose used (1800 γ).

The relation between the rat unit and androsterone activity.

The data referring to the average activity of androsterone and the rat unit are summarised in Table VII. In column I is given the amount of the dose injected in γ . In column II is given the average percentage increase in weight of the organs per 100 γ for doses from 200 to 900 γ . The values for the prostate are very close to those for the prostate with seminal vesicles, both the actual figures

Table VI. *Effect of androsterone on seminal vesicles, penis and preputial glands.*

Organs	Weights	Average percentage increase in weight of organs of rats injected with (γ)						Average percentage increase per 100 γ for doses from 200 to 900 γ
		200	450	600	900	1350	1800	
Seminal vesicles	{Actual	38	112	168	177	236	274	23.0
	{Per unit of body weight	28	132	169	157	215	287	22.6
Penis	{Actual	28	48	71	89	116	93	11.0
	{Per unit of body weight	18	56	72	76	104	93	10.3
Preputial glands	{Actual	58	77	119	132	136	164	18.0
	{Per unit of body weight	45	105	120	116	119	160	18.0

Table VII. *Relation between the average activity of androsterone and the rat unit.*

	I	II	III	IV		V	VI
Organs	Dose actually injected in γ	Average percentage increase per 100 γ for doses from 200 to 900 γ	No. of γ per rat unit	Percentage increase in weight		No. of rat units injected	
				Expected assuming values in column II	Actually obtained		
Prostate		Actual weights.					
	200	24.7	162	49	48	1.2	
	450			111	110	2.8	
	600			148	142	3.7	
	900			222	232	5.6	
	1350			333	277	8.3	
	1800			445	237	11.1	
		Weights per unit of body weight.					
	200	23.7	169	47	39	1.2	
	450			107	121	2.7	
	600			142	145	3.6	
	900			213	205	5.3	
	1350			320	253	8.0	
	1800			427	246	10.7	
	Prostate with seminal vesicles		Actual weights.				
200		24.3	165	49	46	1.2	
450				109	110	2.7	
600				146	145	3.6	
900				219	221	5.5	
1350				328	272	8.2	
1800				437	252	10.9	
		Weights per unit of body weight.					
200		23.3	172	47	36	1.2	
450				105	122	2.6	
600				140	146	3.5	
900				210	197	5.2	
1350				315	245	7.9	
1800				419	253	10.5	

(24.7 and 24.3 % respectively) and those calculated per unit of body weight (23.7 and 23.3 % respectively). There is also little difference between the percentage increase calculated from the actual figures and from the figures per unit of body weight in the case of these two organs, *e.g.* 100 γ gave 24.7 % increase in the actual weight of the prostate and 23.7 % increase when calculated per 200 g. of body weight. From Table VI it can be seen that the respective data

(last column) for the seminal vesicles were 23.0 and 22.6 %, *i.e.* results very close to those for the prostate.

The values of column III are obtained from those of column II and our definition of one rat unit of testicular hormone [Korenchevsky and Dennison, 1934, 2, p. 1498] as that amount which produces 40 % increase in the weight of the prostate alone (for "comb growth" activity) or in the weight of the prostate with seminal vesicles (for "whole male sexual" activity). Again, as in column II, the values in column III are very close, one rat unit of "comb growth" activity being contained in 162–169 γ and of "whole male sexual" activity in 165–172 γ .

In the preliminary communication [Korenchevsky, 1935] an approximate estimation was based on experiments with only a few rats and these values were given as 175 and 179 γ , which are very close to those now given, being obtained from a sufficiently large number of rats to yield reliable figures.

In columns IV and V of Table VII percentage increases actually obtained are compared with those which would be theoretically expected assuming the average value given in column II and a direct proportional relationship between the dose and percentage increase. This comparison shows that the figures actually obtained were very close to these theoretical figures for doses 200–900 γ and that the divergence increases considerably with higher doses.

Column VI gives the amount of androsterone injected as expressed in rat units and shows that the direct proportional relationship between the dose and the effect existed, approximately, from 1 to 6 rat units and that a definite flattening of the curve was observed when 8 or more rat units were injected.

A statistical interpretation of the results obtained in the assay.

With the kind permission of Dr E. S. Pearson we include his opinion as expressed in the following quotation from his letter.

"Let us denote by y the percentage increase in an organ obtained on applying Dr Korenchevsky's procedure to a litter of rats, one group of the litter being treated as a control and the remainder injected with a dose of $x\gamma$. For example, from Table II it is seen that for litter No. 8, $y=192$ %, for $x=900\gamma$. Then the four diagrams show as black spots the average values of y plotted against x . Cubic curves, constrained to pass through the origin, have been fitted to these averages, using the method of least squares and giving all averages equal weight. These curves are useful for graduation purposes, but no great stress should be laid on their precise form, particularly in the region of large doses.

For the range of doses 200–900 γ , it is clear that a good approximation to the assay curve will be obtained by a straight line. The slopes of these lines and their probable errors have been calculated as follows. An examination of Dr Korenchevsky's results shows that, whilst the variation in y from litter to litter increased steadily with x , the variation in the ratio

$$\frac{y}{x} = \frac{\% \text{ increase in organ} \times 1000}{\text{dose}} = z, \text{ say,}$$

remains approximately constant. A useful measure of the reliability of an assay appears, therefore, to be the probable error of z^1 .

¹ In making these calculations it has been tacitly assumed that all the experimental results might be regarded as of equal value, irrespective of the number of rats in the litter. From the statistical point of view this is not completely satisfactory, but if further assays are made with at least 2 control rats and 2 dosed rats per litter, the probable error to be expected will be less than that allowed for in the following discussion.

The following Table VIII shows:

Column 1. Organ and basis of calculation.

Column 2. Average value of z for the 22 groups injected (doses 200, 450, 600 and 900 γ only being considered). This represents in the range 200–900 γ , the average percentage increase in organ per 1000 γ of the drug, and determines the slope of the straight lines shown in the diagrams.

Column 3. Probable error of Dr Korenchevsky's averages given in column 2. These probable errors are $1/\sqrt{22}$ times those given in column 4 (the figure being raised to the next highest integer when a decimal place occurs).

Column 4. Probable error of a value of z determined from a single litter.

Column 5. Probable error of z determined from the average of 3 litters. These figures are $1/\sqrt{3}$ times those given in column 4.

Table VIII. *Statistical summary of results of experiments*
(E. S. Pearson).

(1)	(2)	(3)	(5) Probable error of z if determined	
			(4) From a single litter	(5) From three litters
Organ and basis of calculation	Average z (22 groups of rats)	Probable error of average z (22 groups)		
Prostate { Actual weight	245	± 8 (3 %)*	± 34 (14 %)*	± 20 (8 %)*
{ per 200 g.	233	± 10 (4 %)	± 43 (19 %)	± 25 (11 %)
Seminal vesicles { Actual weight	230	± 13 (6 %)	± 57 (25 %)	± 33 (15 %)
{ per 200 g.	223	± 15 (7 %)	± 69 (31 %)	± 40 (18 %)
Prostate and { Actual weight	241	± 6 (3 %)	± 24 (10 %)	± 14 (6 %)
seminal vesicles { per 200 g.	228	± 9 (4 %)	± 38 (17 %)	± 22 (10 %)

* The figures in brackets are the values of the probable errors expressed as a percentage of the average z of column (2).

The figures given in brackets in columns (3), (4) and (5) are the values of the probable errors expressed as a percentage of the average figure given in column (2), *e.g.* row 1, column 4 gives 34 (14 %), since 34 is 14 % of 245.

The results shown in the table suggest that in the present case somewhat more reliable assays can be made using the actual weights of organs rather than by adjusting these to body weight. They also show that the seminal vesicles alone give more variable results than those for the prostate.

Should an experimenter in further work wish to judge whether the difference between his assay and Dr Korenchevsky's is significant, this may be done as follows: suppose after treating 4 litters with a dose of androsterone, containing, he believes, $x = 600\gamma$, he finds an average increase in prostate (based on actual weights) of $y = 126\%$; this corresponds to a value of $z = \frac{126 \times 1000}{600} = 210$; on the assumption that his technique is as reliable as Dr Korenchevsky's, the probable error of this z based on 4 litters will be $\frac{34}{\sqrt{4}} = 17$ (using the appropriate probable error from row 1, column 4 of the table). Thus he must compare 210 ± 17 with Dr Korenchevsky's 245 ± 8 . The difference is 35 and its probable error

$$\sqrt{(17)^2 + (8)^2} = 19,$$

that is to say, the difference is barely twice the probable error and cannot be regarded as significant."

The effect of androsterone on the weight of the penis and preputial glands.

The average data for these glands are summarised in Table VI. They show an increase in the effect on the glands with an increase in the dose, the effect, however, being much less regular than in the case of the prostate. The flattening of the curve was also noticeable with higher doses.

If we assume the values given in column 3, Table VII of 165 γ (actual figures) and 172 γ (per unit of body weight) for one rat unit of "whole male sexual" activity, the increase in the weight of the penis per rat unit of androsterone will be 18.2 and 17.17 % respectively. A comparison of these figures obtained by androsterone injections with those previously obtained by injections of testicular hormone preparations extracted from human urine [Korenchevsky *et al.*, 1932, pp. 2100–2101], shows a very close similarity in the results which in these earlier experiments were 18 % (actual figures) and 15 % (per unit of body weight) increase in the weight of the penis per rat unit. The response of the preputial glands to the injections is greater than that of the penis (Table VI), 100 γ producing an increase of about 18 % or 30 % per rat unit.

Effect on the other organs investigated.

In addition to the effect on the sexual organs an investigation was made of the changes in weight of the thymus, adrenals, thyroid and intra-abdominal fat.

The only changes observed were a decrease in most cases in the weight of the thymus and the adrenal glands of 10–30 % both in actual figures and those per unit of body weight.

DISCUSSION.

A saturated solution of androsterone in olive oil contains about 1 % of androsterone when, as far as possible, all traces of alcohol, the intermediate solvent, are removed from the oil *in vacuo*; *i.e.* androsterone is not very soluble in oil. The results of our assay, therefore, apply only to the oily solution of the hormone free from alcohol, since it is probable that the use of other solvents or the non-removal of all or part of the intermediate solvent would result in a different rate or degree of absorption of the hormone from the subcutaneous tissue, giving an apparently different activity for the preparation. We injected all doses as the same quantity (0.2 ml. per day) of solutions of different concentrations. It is possible that different results would be obtained by using varying quantities of a solution of one concentration only. These points are a matter for further investigation.

By our method, the part of the curve corresponding to doses of 200–900 γ was found to be suitable for the assay of the "comb growth" activity and the "whole male sexual" activity of androsterone ("assayable part of curve"). As we had found in our previous experiments with the testicular hormone preparations extracted from human urine, the rat units of these two forms of activity proved to be nearly identical, owing to the fact that both these hormones had about the same quantitative effect on the prostate alone as they had on the seminal vesicles alone.

In addition to this similarity it was found that on the average the effects on the penis of the natural preparations used and of the artificial hormone were also nearly the same.

The direct proportional relationship between the dose and the effect, within certain limits of the dose, and the approximate equality of the rat and capon units were also points of similarity between the natural and artificial preparations.

One difference must be considered here, the flattening of the curve with the

higher doses of androsterone, which we were unable to obtain with 8 c.u. of the "natural" hormone preparation. This is perhaps related to different rates of absorption from nearly saturated solutions. Our present results, however, cannot be compared with any of the "natural" preparations we received from various firms, since:

- (1) intermediate solvents may not have been completely removed;
- (2) the preparations may have contained other testicular hormones capable of increasing the effect of androsterone.

We have suggested several times in our previous papers that there is probably more than one testicular hormone present in the organism.

As an *international unit of androsterone* in pure oily solution that dose is preferable which will give the smallest definite physiological effect. We have found for the prostate and prostate with seminal vesicles this dose to be that which produces 40 % increase in the weight, actual or per unit of body weight. Therefore, from our results, taking into account that the probable error of our present results does not seem to be more than ± 5 %, 170 γ of androsterone might be suggested as one rat unit, or alternatively, since one capon unit has been found to vary from 150 to 200 γ of androsterone, either of these round figures might be more suitable, being applicable to both methods. In our previous papers we suggested that the assay should be made with at least 3 litters, provided that there are at least 2 rats in each group. The present experiments show (Table VIII, column 5), that with 3 litters the probable error would be less than ± 12 %, which for practical purposes, is quite satisfactory.

Up to the present, we have published all our results expressed both as actual weights and the weights per unit of body weight, since they serve as a check, the one to the other and there are arguments for and against both methods of calculation. We ourselves feel confident in the reliability of the results only when the general averages expressed as percentage changes by the two methods are as close as they are for instance in the present or our previous papers.

Dr Pearson finds that the actual results are less variable than those calculated per unit of body weight. That this could be so, can best be explained by the following example of groups I (litter-mates of similar weight) and II (litter-mates of dissimilar weight) (see Table below) taken from the present experiments of the assay of androsterone.

No. of group	No. of litter-mate	Body weight	Weight fat (g.)		Weight of prostate (mg.)	
			actual	per 200 g.	actual	per 200 g.
I	{ 1	237	6.6	5.6	211	178
	{ 2	215	6.8	6.3	176	164
II	{ 3	232	9.7	8.4	195	168
	{ 4	170	3.0	3.5	219	258

If the deposition of fat in the rats does not differ greatly, the two values, actual and per 200 g. of body weight, are very close and the weights of the sexual organs seem to be approximately proportional to the body weight (see group I). If the deposition of fat in the rats is very different, so that the weight of the rat is considerably influenced by it, the weights of the sexual organs may be close in actual figures and become, it seems fallaciously, very different per unit of body weight (see group II). Probably physiological variations in the size of the rats are of different origin from those produced by pathological factors, which factors however are often not detectable at autopsy. Consequently the exclusion of a suspicious (because irregular) rat from the results is not justified.

SUMMARY.

1. Pure crystalline testicular hormone, prepared by Ruzicka, was dissolved in olive oil and assayed by the author's method on 74 castrated male rats.

2. A relation was found to exist between the dose and the effect of androsterone on the secondary sexual organs, this relation being investigated statistically and represented graphically in the case of the prostate and the prostate with seminal vesicles.

3. The "assayable" part of the curve for these organs for the purposes of assay may be represented between the doses 200 and 900 γ ("assayable" doses) by a straight line.

4. One rat unit of both "comb growth" activity and "whole male sexual" activity was found on the average to be contained in approximately 170 γ , which is also approximately the same amount as that found by other workers for the capon unit.

5. This figure, or the round number 150 or 200 γ , is suggested as one international unit of androsterone, being suitable both for the assay by the capon method and by our rat method.

6. The probable error of our value of the rat unit of androsterone is less than $\pm 5\%$, if all 11 litters injected with assayable doses are taken into consideration.

7. The points of similarity and dissimilarity between androsterone and the purified preparations of the hormone extracted from human urine have been discussed.

8. In making an assay, the results obtained from the actual weights should be checked by those calculated per unit of body weight and the two results should be in close agreement, when expressed as the percentage changes in the weights of the organs.

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