CCLIV. THE EFFECTS OF WATER-SOLUBLE PREPARATIONS OF ANDROSTERONE AND ANDROSTERONE-DIOL ON CASTRATED RATS.

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RUZICKA *et al.* [1935] prepared a monosuccinic ester of androsterone, of which Prof. Ruzicka sent to us two water-soluble preparations for investigation: (1) the sodium salt and (2) the lithium salt. In the following they will be designated as "w.-s. androsterone". We also received from Prof. Ruzicka a lithium salt of the monosuccinic ester of androsterone-diol, which will be designated as "w.-s. diol."

Tschopp [Ruzicka *et al.*, 1935, p. 213], using his method of injecting capons once a day for 6 days, found that 1 c.u. was contained in 80–100 γ of w.-s. androsterone and (according to a personal communication) in about 200 γ of w.-s. diol.

As in our preliminary experiments the activity of w.-s. diol on castrated rats was found to be much greater than that of w.-s. androsterone, most of the experiments were performed with the diol salt; some of the experiments were carried out with the lithium salt of w.-s. androsterone. The results of these experiments are given in the present paper.

Technique.

The salts were received in solutions of known strength. They were distributed in ampoules and steamed for 30 min. on 3 consecutive days. Dilutions were prepared with distilled water, which in some preparations caused opalescence and the formation of a small amount of precipitate; this however did not interfere with the accuracy of the test. Before taking the solution into the syringe, care was taken to suspend the precipitate in the solution by stirring it well.

The solutions were injected into the animals in quantities of 0.1–0.2 ml. per injection, each 0.1 ml. being injected into a separate place under the skin.

Whilst in our experiments oily solutions were always injected twice daily, the water-soluble preparations were injected three times daily, between 9.30 and 10 a.m., at 3 p.m. and between 9 and 11 p.m. This was done because it is well known that water-soluble hormones are usually quickly absorbed from the subcutaneous tissue and quickly excreted or perhaps inactivated in the organism.

Since, in our previous papers on assays, we have given a sufficient number of details in the form of tables, some of the detailed tables are omitted from this paper in order to economise space.

Fig. 1. Relation between dose of water-soluble diol and percentage increase in actual weight of prostate and of seminal vesicles.

Fig. 2. Relation between dose of water-soluble diol and percentage increase in weight per unit body weight of prostate and of seminal vesicles.

I. *Water-soluble diol.*

Assay. Experiments were performed on 71 rats, belonging to 13 litters, castrated 21–26 days after birth.

Seven days of injection were started 30–38 days after castration. The weights of the rats varied comparatively little, being between 183 and 234 g. at the end of the experiment. Daily doses of 97, 194, 389, 777, 1555 and 3109 γ were injected, the total number of rats in each of the groups injected with these doses being 10, 6, 9, 8, 9 and 4 respectively. The total number of control rats was 25. The technique of assay was the same as that used in the previous paper.

Effect on the secondary sexual organs. w.-s. diol had a considerably greater effect on the seminal vesicles than on the prostate as is shown by Tables I and II

Table I. *Influence of w.-s. diol on seminal vesicles.*

The average weights, actual and calculated per 200 g. of body weight, of the seminal vesicles of the rats in each group of each litter and their percentage increase after the injections.

No of litter	Control rats	Rats injected with γ											
		97	194	389	777	1555	3109	97	194	389	777	1555	3109
		A. Actual weights, mg.						% increase					
1	13	—	—	46	66	108	—	—	—	254	408	731	—
2	14	—	—	46	—	89	—	—	—	229	—	536	—
3	16	—	—	—	55	—	—	—	—	—	244	—	—
4	13	—	—	45	—	101	—	—	—	246	—	677	—
5	13	—	—	—	68	—	128	—	—	—	423	—	884
6	12	—	—	34	—	99	—	—	—	183	—	725	—
7	10	—	—	—	59	—	185	—	—	—	490	—	1750
8	13	25	—	—	—	—	—	92	—	—	—	—	—
9	12	22	—	—	—	—	—	83	—	—	—	—	—
10	11	20	27	—	—	—	—	82	147	—	—	—	—
11	10	20	25	—	—	—	—	100	150	—	—	—	—
12	10	22	31	—	—	—	—	120	210	—	—	—	—
13	10	—	30	—	—	—	—	—	200	—	—	—	—
Average	12	22	28	43	62	99	157	95	177	228	391	667	1317
		B. Weights per 200 g. body weight, mg.						% increase					
1	12	—	—	50	69	107	—	—	—	317	475	791	—
2	13	—	—	43	—	95	—	—	—	231	—	631	—
3	15	—	—	—	49	—	—	—	—	—	227	—	—
4	12	—	—	41	—	79	—	—	—	242	—	558	—
5	12	—	—	—	57	—	110	—	—	—	375	—	817
6	12	—	—	34	—	98	—	—	—	183	—	734	—
8	10	—	—	—	53	—	127	—	—	—	430	—	1170
9	11	21	—	—	—	—	—	91	—	—	—	—	—
10	11	20	—	—	—	—	—	82	—	—	—	—	—
11	12	22	28	—	—	—	—	83	133	—	—	—	—
12	10	19	27	—	—	—	—	90	170	—	—	—	—
13	10	22	30	—	—	—	—	120	200	—	—	—	—
13	10	—	30	—	—	—	—	—	200	—	—	—	—
Average	12	21	29	42	57	95	119	93	176	243	377	681	994

and Figs. 1 and 2. Therefore the effect on the prostate with seminal vesicles was also greater than that on the prostate alone (Table III, Fig. 3). The action on the penis and preputial glands was much less (Tables III and IV). The penis however gave a comparatively regular response to the different doses (see Figs. 4 and 5), whilst in the case of the preputial glands there was no such regularity.

The rat unit. Taking that part of the curve of the average percentage increase in the weight of the prostate between 0 and 194 γ to be a straight line, 40% increase, i.e. one rat unit of "comb-growth activity" would be contained in

Table II. *Influence of w.-s. diol on prostate.*

The average weights, actual and calculated per 200 g. of body weight, of the prostate of the rats in each group of each litter and their percentage increase after the injections.

No. of litter	Control rats	Rats injected with γ											
		97	194	389	777	1555	3109	97	194	389	777	1555	3109
		A. Actual weights, mg.						% increase					
1	66	—	—	140	169	280	—	—	—	112	156	324	—
2	52	—	—	161	—	293	—	—	—	210	—	453	—
3	62	—	—	—	219	—	—	—	—	—	253	—	—
4	54	—	—	176	—	299	—	—	—	226	—	454	—
5	50	—	—	—	197	—	332	—	—	—	294	—	564
6	55	—	—	156	—	277	—	—	—	184	—	403	—
7	51	—	—	—	230	—	320	—	—	—	351	—	527
8	58	92	—	—	—	—	—	58	—	—	—	—	—
9	54	97	—	—	—	—	—	80	—	—	—	—	—
10	55	79	110	—	—	—	—	44	100	—	—	—	—
11	50	84	113	—	—	—	—	68	126	—	—	—	—
12	50	73	116	—	—	—	—	46	132	—	—	—	—
13	51	—	105	—	—	—	—	—	106	—	—	—	—
Average	54	85	111	158	204	287	326	59	116	183	264	409	546
		B. Weights per 200 g. body weight, mg.						% increase					
		97	194	389	777	1555	3109	97	194	389	777	1555	3109
		A. Actual weights, mg.						% increase					
1	62	—	—	154	177	276	—	—	—	148	186	345	—
2	51	—	—	152	—	303	—	—	—	198	—	498	—
3	60	—	—	—	196	—	—	—	—	—	227	—	—
4	53	—	—	155	—	261	—	—	—	192	—	392	—
5	46	—	—	—	168	—	284	—	—	—	265	—	517
6	54	—	—	160	—	275	—	—	—	196	—	409	—
7	49	—	—	—	182	—	302	—	—	—	272	—	516
8	50	80	—	—	—	—	—	60	—	—	—	—	—
9	51	92	—	—	—	—	—	80	—	—	—	—	—
10	61	86	117	—	—	—	—	41	92	—	—	—	—
11	48	80	122	—	—	—	—	68	154	—	—	—	—
12	48	72	114	—	—	—	—	50	137	—	—	—	—
13	49	—	104	—	—	—	—	—	112	—	—	—	—
Average	52	82	114	155	181	279	293	60	124	184	238	411	517

Fig. 3. Relation between dose of water-soluble diol and percentage increase in actual weight of prostate weighed together with seminal vesicles.

about 67 γ . The rat unit of the "whole male sexual activity" (40% increase in the weight of the prostate with seminal vesicles) would be contained in about 64 γ . These amounts of the water-soluble salt contain about 50 and 48 γ of pure diol respectively.

Table III. *Effect of w.-s. diol on prostate weighed with seminal vesicles and on preputial glands.*

Organs	Weights	Average % increase in weight of organs of rats injected with γ					
		94	194	389	777	1555	3109
Prostate with seminal vesicles	{ Actual	66	125	195	290	459	638
	{ Per unit body weight	66	134	194	263	457	609
Preputial glands	{ Actual	—	63	58	88	111	188
	{ Per unit body weight	—	73	55	89	111	176

Table IV. *Influence of w.-s. diol on penis.*

The average weights, actual and calculated per 200 g. of body weight, of the penis of the rats in each group of each litter and their percentage increase after the injections.

No. of litter	Control rats	Rats injected with γ											
		94	194	389	777	1555	3109	94	194	389	777	1555	3109
		A. Actual weights, mg.						% increase in weight					
1	115	—	—	175	195	244	—	—	—	52	70	112	—
2	94	—	—	177	—	210	—	—	—	88	—	123	—
3	90	—	—	—	209	—	—	—	—	—	132	—	—
4	90	—	—	170	—	227	—	—	—	89	—	152	—
5	90	—	—	—	198	—	237	—	—	—	120	—	163
6	91	—	—	156	—	232	—	—	—	72	—	155	—
7	79	—	—	—	162	—	209	—	—	—	105	—	165
8	78	106	—	—	—	—	—	36	—	—	—	—	—
9	76	106	—	—	—	—	—	40	—	—	—	—	—
10	74	111	133	—	—	—	—	50	80	—	—	—	—
11	74	105	123	—	—	—	—	42	66	—	—	—	—
12	74	101	116	—	—	—	—	36	57	—	—	—	—
13	76	—	117	—	—	—	—	—	54	—	—	—	—
Average	86	106	122	170	191	203	223	41	64	75	107	136	164
		B. Weights per 200 g. body weight, mg.						% increase					
1	108	—	—	188	204	243	—	—	—	74	89	125	—
2	92	—	—	166	—	221	—	—	—	80	—	140	—
3	87	—	—	—	187	—	—	—	—	—	115	—	—
4	88	—	—	149	—	146	—	—	—	69	—	66	—
5	83	—	—	—	164	—	203	—	—	—	98	—	145
6	90	—	—	160	—	230	—	—	—	78	—	156	—
7	80	—	—	—	146	—	196	—	—	—	83	—	145
8	68	92	—	—	—	—	—	35	—	—	—	—	—
9	71	100	—	—	—	—	—	41	—	—	—	—	—
10	82	120	142	—	—	—	—	46	73	—	—	—	—
11	71	95	133	—	—	—	—	37	87	—	—	—	—
12	71	99	114	—	—	—	—	39	61	—	—	—	—
13	72	—	116	—	—	—	—	—	61	—	—	—	—
Average	82	101	126	166	175	210	200	40	71	75	96	122	145

Effect on the other organs and tissues. Changes of less than 10% in the weights of the organs were not taken into consideration. There was no change in the gain in body weight during the period of the injections. Of the 14 groups injected there was a slight increase in the fat deposition in 10, as judged by the increase in weight of the intra-abdominal fat (13–66%). In most groups (9 out of 14) the adrenals decreased slightly (11–36%) and thyroid increased slightly (10–52% in 8 out of 14 groups). There was also a decrease in the weight of the thymus in most groups (13–38% in 9 groups).

Fig. 4. Relation between dose of water-soluble diol and percentage increase in actual weight of penis.

Fig. 5. Relation between dose of water-soluble diol and percentage increase in weight per unit of body weight of penis.

The hypophysis was examined in only six litters and in five of the litters the hypophysis of the rats injected with diol decreased by 10–27 %.

Although all the changes which have been mentioned above were small and not invariably present, the direction of the changes was specific for the respective organs of castrated rats, in which the injection of testicular hormone is causing a return towards the normal condition.

No changes were found in the weight of the liver, kidneys, spleen or heart.

II. Water-soluble androsterone.

Assay. Experiments were performed on 28 rats belonging to five litters, which were similar in age, weight and age at castration to those used in the diol assay. The numbers of rats used in the different groups were: control rats 9, rats injected with 1993, 3986, 7971, 11957 and 15942 γ —9, 6, 5, 3, 3 and 2 respectively.

The effect of water-soluble androsterone on the secondary sexual organs and a comparison with the effect of pure androsterone, dissolved and injected in oil. The

Table V. *Influence of w.-s. androsterone on prostate and on seminal vesicles.*

The average weights, actual and calculated per 200 g. of body weight, of the prostate of the rats in each group and their percentage increase after injections.

No. of litter	Control rats	Rats injected with γ									
		1993	3986	7971	11957	15942	1993	3986	7971	11957	15942
<i>Prostate.</i>											
A. Actual weights, mg.						% increase in weight					
14	52	74	—	—	—	—	42	—	—	—	—
15	53	75	—	—	154	—	42	—	—	190	—
16	53	73	—	—	194	—	38	—	—	266	—
17	49	—	95	—	—	181	—	94	—	—	270
18	49	—	107	151	—	—	—	118	208	—	—
Average	51	74	101	151	172	181	41	106	208	228	270
B. Weights per 200 g. body weight, mg.											
						% increase					
14	48	71	—	—	—	—	48	—	—	—	—
15	50	72	—	—	153	—	44	—	—	206	—
16	50	79	—	—	197	—	58	—	—	294	—
17	46	—	84	—	—	166	—	83	—	—	261
18	48	—	107	148	—	—	—	123	208	—	—
Average	48	74	96	148	175	166	50	103	208	250	261
<i>Seminal vesicles.</i>											
A. Actual weights, mg.						% increase					
14	12	13	—	—	—	—	8	—	—	—	—
15	11	16	—	—	40	—	45	—	—	264	—
16	11	15	—	—	47	—	36	—	—	327	—
17	12	—	27	—	—	44	—	125	—	—	266
18	12	—	18	24	—	—	—	50	100	—	—
Average	12	15	23	24	44	44	30	88	100	296	266
B. Weights per 200 g. body weight, mg.											
						% increase					
14	11	13	—	—	—	—	18	—	—	—	—
15	11	16	—	—	40	—	45	—	—	264	—
16	11	16	—	—	48	—	45	—	—	336	—
17	11	—	24	—	—	41	—	118	—	—	273
18	11	—	18	30	—	—	—	64	173	—	—
Average	11	15	21	30	44	41	36	91	173	300	273

curve (not given here) representing the percentage increase in the weight of the prostate with different doses approximated to a straight line with the smaller doses, *i.e.* between 1993 and 7971 γ , after which there was a definite flattening of the curve (Table V). This also occurred with the pure preparation [Korenchevsky and Dennison, 1935, 1, Figs. 1 and 2].

The greatest average percentage increase in the weight of the prostate obtained with the water-soluble preparation (270 % actual weight) was also nearly the same as the highest average value given by the fat-soluble preparation [277 %; Korenchevsky and Dennison, 1935, 1, Table II, dose 1350 γ].

The reactions of the seminal vesicles were also similar with the two preparations, the percentage increase being in most cases equal to or a little less than that of the prostate (though occasionally with some doses the increase of the seminal vesicles was somewhat larger than that of the prostate). Therefore the average figures of the ratio of the prostate to seminal vesicles were greater than 100 for both preparations (Table VIII). The greatest average percentage increases obtained in the weights of the seminal vesicles with the two preparations were also similar, being 287 % with fat-soluble androsterone [Korenchevsky and Dennison, 1935, 1, Table VI] and 300 % with w.-s. androsterone.

This same similarity in maximum reaction to the two preparations was also seen with the penis, in which the greatest average percentage increase was 116 % with the fat-soluble and 110 % with the water-soluble preparation. This organ also showed the same flattening of the curves of percentage increase in weight with the higher doses (see Table VI, this paper, and Table VI, Korenchevsky and Dennison [1935, 1]).

Table VI. *Effect of w.-s. androsterone on prostate weighed with seminal vesicles, penis, preputial glands and thymus.*

Organs	Weights	Average % change in weight of organs of rats injected with γ				
		1993	3986	7971	11957	15942
Prostate with seminal vesicle	{ Actual	+35	+103	+198	+240	+269
	{ Per unit body weight	+40	+100	+202	+259	+263
Penis	{ Actual	+17	+54	+43	+95	+107
	{ Per unit body weight	+23	+51	+46	+110	+107
Preputial glands	{ Actual	+32	+74	+69	+94	+76
	{ Per unit body weight	+37	+72	+68	+112	+73
Thymus	{ Actual	-15	-24	-14	-33	-28
	{ Per unit body weight	-11	$\pm$ 0	-9	-28	-28

The reaction of the preputial glands was less with the water-soluble than with the fat-soluble preparation (see the same tables), the largest figures of percentage increase being 112 and 164 respectively. The reaction of the preputial glands however is always so irregular that it is not possible to attach much importance to this difference.

With nearly all the doses used the weight of the thymus decreased slightly (Table VI). There was no change in the fat deposition. The other organs were not investigated in these experiments.

The rat unit of w.-s. androsterone. The curve of the percentage increase in the weight of the prostate over the range 0-7971 γ approximates to a straight line, *i.e.* for this range a direct proportional relationship between the dose and the effect may be assumed. This is seen particularly clearly in the average figures per 200 g. of body weight (Table V). It therefore seems to be justifiable

to calculate the rat unit from the average of the effects produced by the doses within this range, *i.e.* by 1993, 3986 and 7971 γ . In this case one rat unit of "comb-growth activity" is contained in about 1.6 mg. and one rat unit of "whole male sexual activity" in about 1.7 mg. These quantities of the water-soluble androsterone salt contain about 1.19 and 1.21 mg. of pure crystalline androsterone.

Since one rat unit was contained in about 170 γ of pure androsterone dissolved in oil [Korenchevsky and Dennison, 1935, 1], we must conclude that androsterone administered as the pure substance dissolved in oil is more active than the water-soluble salt of the ester.

Statistical description of the curves. J. M. C. Scott, under direction of Prof. E. S. Pearson (Department of Statistics, University College), has represented graphically in the form of the above curves the results of the assay, and the same statistical method as used in the previous paper [Korenchevsky and Dennison, 1935, 2] was again employed.

DISCUSSION.

The comparison of the effects of water-soluble diol and fat-soluble diol.

Since each of the preparations used was injected in different doses, it is not easy to make a comparison of the two preparations. Some of the figures in the tables of the previous and present papers however provide suitable data, and other data are summarised in Table VII, in which only the actual weights are tabulated since they differed little from those per unit of body weight.

Table VII. *Comparison of the effect of w.-s. diol* with that of fat-soluble diol.*

Preparations of diol	Doses* injected γ	% increase in weight of organs per 100 γ of the injected doses				
		Seminal vesicles	Prostate	Prostate + seminal vesicles	Penis	Preputial glands
1. Fat-soluble	100	189	117	127	84	54
2. Water-soluble*	100	124	79	87	49	—
3.)	25	320	192	212	140	—
4.)	50	232	178	186	124	18
5.) Fat-soluble	100	189	117	127	84	54
6.)	200	150	88	98	51	39
7.)	350	90	57	61	29	23
8.)	74	128	80	89	55	—
9.)	148	120	78	84	43	43
10.) Water-soluble*	295	77	62	66	25	20
11.)	580	67	46	50	18	15
12.)	1161	57	35	40	12	10
13.)	2321	57	24	27	7	8
14. Fat-soluble	{ Av. for doses 25-200	223	144	156	100	37
15. Water-soluble*	Av. for all doses	84	54	59	27	19

* Doses for w.-s. diol are given as the amount of cryst. diol contained in the water-soluble salt.

(1) It can be seen from Tables II to IV of the previous paper that in all the organs a sharp flattening of the curve occurs after the injection of 200 γ of fat-soluble diol, whilst after the injection of the water-soluble salt containing 2321 γ of pure diol (the highest dose used) sharp flattening had still not been reached.

Thus, with 350 γ of "fat-sol. diol" the percentage increases in the actual weights of the seminal vesicles, prostate and penis were 314, 198 and 102 respectively and with 2321 γ of diol in the "w.-s. salt" were 1317, 546 and 164 respectively.

With the fat-soluble preparation therefore a very pronounced flattening of the curves occurs with much smaller doses than with the water-soluble preparation. This explains why it is possible to obtain a much greater increase in the weight of the atrophied organs by the injection of the water-soluble preparation than with the fat-soluble preparation.

(2) The stimulation of the growth of the organs with both the fat-soluble (lines 3-7, Table VII) and the water-soluble (lines 8-13, Table VII) preparations becomes less per unit (100 γ) of the preparation as the dose is increased.

The fat-soluble preparation however produces a greater effect per 100 γ than the water-soluble preparation (compare lines 3-7 with lines 8-13, Table VII).

This difference is seen particularly clearly if similar doses are chosen. Since the doses 74 and 148 γ (lines 8 and 9, Table VII) of w.-s. diol produce nearly the same effect per unit dose, this effect would presumably be produced by all doses between 74 and 148 γ , including the dose 100 γ , the probable effect of which is interpolated in line 2 of Table VII. The dose 100 γ of fat-soluble diol was actually used for injection (line 1, Table VII). Comparison of the effects of 100 γ of the two preparations shows that the fat-soluble preparation produces a considerably greater effect than that produced by the water-soluble preparation.

In spite of this, however, the increase in the effect by increasing the large doses and thus producing the largest possible reaction was, as stated in the previous paragraph, more pronounced in the case of the water-soluble preparation. This is of course only true for the concentrations of fat-soluble diol used.

(3) The two previous sections have described the differences between the water-soluble and fat-soluble preparations. The points of similarity between the two substances have already been mentioned in this and the previous papers. With both preparations the "effect per 100 γ " decreases as the dose increases and the stimulation of the growth of seminal vesicles is greater than that of the prostate.

The difference between the actions of diol and of androsterone.

As far as can be judged from experiments of 7 days of injection, the chief difference between androsterone and diol is that androsterone does not stimulate the growth of the prostate and seminal vesicles in a normal relation, as is done by diol. This is true for both the fat-soluble and water-soluble preparations.

The seminal vesicles atrophy after castration to a much greater extent than the prostate, the average weights of the seminal vesicles and prostate 30 days after castration being about 10-12 mg. and 50-55 mg. respectively. Since the weights of these two glands are approximately equal in the normal adult rat, it is clear that, if both these structures are to be restored to the normal condition, injections of the hormone should produce a greater percentage increase in the weight of the seminal vesicles than in that of the prostate.

The ratios of $\frac{\% \text{ increase of prostate}}{\% \text{ increase of seminal vesicles}} \times 100$ for both water-soluble and fat-soluble preparations of androsterone and diol are given in Table VIII. It can be clearly seen from this table, that whilst for androsterone on the average this ratio is greater than 100, for diol it is about 60, *i.e.* whilst androsterone stimulates the growth of the seminal vesicles and prostate equally in most of the rats and

Table VIII. *Ratio ($\times 100$) of percentage increase in weight of prostate to that of seminal vesicles after injection of different doses of fat-soluble and water-soluble preparations of androsterone and androsterone-diol.*

Androsterone						Androsterone-diol					
Fat-soluble			Water-soluble			Fat-soluble			Water-soluble		
Ratio prostate to seminal vesicles			Ratio prostate to seminal vesicles			Ratio prostate to seminal vesicles			Ratio prostate to seminal vesicles		
Doses injected γ	Actual weights	Weights per unit body weight	Doses injected γ	Actual weights	Weights per unit body weight	Doses injected γ	Actual weights	Weights per unit body weight	Doses injected γ	Actual weights	Weights per unit body weight
200	126	139	1993	139	149	25	60	58	97	62	65
450	94	92	3986	120	113	50	77	68	194	66	70
600	84	86	7971	208	120	100	62	65	389	80	76
900	131	131	11957	77	83	200	59	58	777	68	63
1350	117	118	15942	102	96	350	63	64	1555	61	60
1800	90	86	—	—	—	—	—	—	3109	41	52
Average	106	109	—	129	112	—	64	63	—	63	63

in some has even more effect on the prostate than on the seminal vesicles, diol produces a greater effect on the seminal vesicles than on the prostate. Thus diol produces a return towards the normal ratio in the development of these glands, whilst androsterone is unable to do so. In 7-day experiments maximum doses both of w.-s. and f.-s. preparations of androsterone produced about the same effects in spite of great quantitative difference of these doses; whilst the maximum dose of diol in w.-s. form had greater action than that of f.-s. preparation.

These dissimilarities in the reaction of the seminal vesicles and in the values of the rat units are the chief differences between the androsterone and diol preparations. As the reaction of the seminal vesicles is not always so regular as that of the prostate, the introduction of one more rat unit (measuring the activity in producing growth of the seminal vesicles) would present difficulties.

On the other hand the unit of "whole male sexual activity" does not demonstrate sufficiently sharply this important difference between the reaction of the seminal vesicles and of the prostate.

Therefore in assays made by our method we suggest the additional use of the ratio—percentage increase in weight of prostate to that of the seminal vesicles—which, considered in conjunction with the size of the rat units, may serve as an important point for differentiating between the two substances.

The difference in the effects on capons and on castrated rats.

As was stated at the beginning of the paper, Tschopp found that the activity of water-soluble androsterone (c.u. = 80–100 γ) on the comb-growth of capons was about twice that of water-soluble diol (c.u. = about 200 γ).

As compared with this ratio the reaction of castrated rats was found to be entirely different, both quantitatively and qualitatively. We found that one rat unit was contained in about 1600 γ of w.-s. androsterone and in 67 γ of w.-s. diol, *i.e.* diol was about 24 times more active than androsterone. Therefore with these two preparations, the reactions of capons (Tschopp's values) and rats show an inverse relationship.

Since the human being is a mammal, the importance of making an assay on mammals (in particular on rats) in addition to the comb-growth assay is thus emphasised. Sooner or later the results obtained with birds must show differences from those on mammals and of this the above is a good example.

SUMMARY.

1. The water-soluble lithium salt of the monosuccinic ester of androsterone and the water-soluble lithium salt of the monosuccinic ester of androsterone-diol were assayed on 99 rats belonging to 18 litters, and the effects of these substances compared with those of pure androsterone and pure diol dissolved in oil.

2. The chief effects of the water-soluble and fat-soluble preparations of androsterone were similar, as was also the case with the two preparations of diol.

3. The chief point of similarity in the androsterone preparations was that the average effect on the prostate was about the same or a little greater than that on the seminal vesicles. The average ratio ($\times 100$) of the increase in the prostate to that of the seminal vesicles (actual weights) was 106 for the fat-soluble and 129 for the water-soluble preparation.

4. The main points of similarity between the diol preparations are (i) the greater quantitative effect as compared with the androsterone preparations and (ii) the fact that they stimulate the growth of the seminal vesicles more than that of the prostate, the average ratio ($\times 100$) of the percentage increase in the prostate to that of the seminal vesicles (actual weights) being 64 for fat-soluble and 63 for water-soluble diol.

5. Therefore, in addition to the difference in the value of the rat units, the difference in this ratio must be considered as one of the important points distinguishing diol from androsterone in assay experiments on rats.

6. The rat unit of "comb-growth activity" was contained in about 1600 γ of water-soluble androsterone and in about 67 γ of water-soluble diol.

7. Whilst the ratio of the rat unit of pure androsterone to the rat unit of the water-soluble salt of the ester was about 1/9, the ratio for the corresponding diol preparations was about 1/3.

8. Whilst on capons it was found that water-soluble androsterone was about twice as active as water-soluble diol, in the present experiments on rats diol proved to be about 24 times more active than androsterone.

9. Other points of similarity and difference between the preparations of androsterone and androsterone-diol were discussed.

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