CCLIII. THE ASSAY OF FAT-SOLUBLE ANDROSTERONE-DIOL.

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From the Lister Institute.

(Received July 24th, 1935.)

THIS paper is a continuation of the investigation which was started at the request of Prof. Ruzicka in order to study the physiological and possible pathological properties of his preparations of male sexual hormones.

Tscherning as reported by Butenandt [1934] and Ruzicka *et al.* [1935] prepared a diol ($C_{19}H_{32}O_2$) from androsterone. The activity of this substance on the comb growth of capons was about three times that of androsterone. From Tschopp's experiments [Ruzicka *et al.* 1935, p. 213] one capon unit was found to be contained in about 15–20 γ of diol (6 days of injection, Tschopp's method), or in 50–60 γ (2 days of injection, Butenandt's method), or in 33 γ by Callow and Deanesly [1935]. The following experiments give the result of the assay on castrated rats of this diol received from Ruzicka.

Technique.

We shall call this diol preparation which is soluble in fat solvents and in oil "fat-soluble diol" in order to distinguish it from the esters of diol from which it is possible to prepare watery solutions.

The experiments were performed on 49 rats belonging to 10 litters. The number of rats, their age and average final weights are given in Table I.

Table I. *Fat-soluble diol.*

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

No. of litter	No. of rats in litter	Age at castra- tion days	No. of days after castra- tion injection started	Weights of rats					
				Control rats	Rats injected with γ				
					25	50	100	200	350
1	5	24	29	188	—	—	200	211	—
2	5	25	31	226	—	219	—	—	—
3	6	25	31	183	—	196	—	200	—
4	5	25	31	189	—	—	202	—	—
5	5	23	31	202	—	—	—	226	—
6	6	23	31	197	—	—	196	—	199
7	4	25	29	209	—	—	—	—	208
8	5	26	30	214	220	—	—	—	—
9	4	26	30	217	192	—	—	—	—
10	4	26	30	184	208	—	—	—	—

The solution of diol in olive oil was prepared in the same way as the solution of androsterone [Korenchevsky and Dennison, 1935]. We found that even with a 0.2% solution of diol in oil a few crystals were deposited on standing after the ampoules had been opened and part of the solution used. Therefore the

highest concentration used in our assay was 0.175% (350 γ in 0.2 ml. daily) at which concentration no crystals could be detected in the solution, even when kept for a considerable time.

*Effect on the seminal vesicles, prostate and
prostate with seminal vesicles.*

As can be seen from Tables II and III and Figs. 1 and 2 the effect was considerably greater on the seminal vesicles than on the prostate. Therefore the weight of the prostate and seminal vesicles together showed a larger effect than that given by the prostate alone (Table V, Fig. 3). There was no very definite difference between the results obtained from the actual weights and the weights per unit of body weight.

Table II. *Influence of fat-soluble 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 injection.

No. of litter	Control rats	Rats injected with γ									
		25	50	100	200	350	25	50	100	200	350
		A. Actual weights, mg.					% increase				
1	10	—	—	29	43	—	—	—	190	330	—
2	13	—	25	—	—	—	—	92	—	—	—
3	10	—	24	—	42	—	—	140	—	320	—
4	10	—	—	29	—	—	—	—	190	—	—
5	10	—	—	—	35	—	—	—	—	250	—
6	8	—	—	23	—	35	—	—	187	—	337
7	10	—	—	—	—	39	—	—	—	—	290
8	11	20	—	—	—	—	82	—	—	—	—
9	12	19	—	—	—	—	58	—	—	—	—
10	10	20	—	—	—	—	100	—	—	—	—
Average	10	20	25	27	40	37	80	116	189	300	314
		B. Calculated per 200 g. of body weight, mg.					% increase				
1	11	—	—	29	45	—	—	—	164	309	—
2	11	—	23	—	—	—	—	109	—	—	—
3	10	—	24	—	42	—	—	140	—	320	—
4	11	—	—	28	—	—	—	—	154	—	—
5	10	—	—	—	33	—	—	—	—	230	—
6	8	—	—	23	—	35	—	—	187	—	337
7	10	—	—	—	—	38	—	—	—	—	280
8	11	19	—	—	—	—	73	—	—	—	—
9	11	20	—	—	—	—	82	—	—	—	—
10	11	19	—	—	—	—	73	—	—	—	—
Average	10	19	24	27	40	37	76	125	168	286	309

The rat unit of diol. In agreement with our definition [Korenchevsky and Dennison, 1934, p. 1498], since 25 γ produced 48% increase in the actual weight of the prostate and 44% increase in the weight per unit of body weight, one rat unit of "comb growth activity" is contained on the average in about 21 γ , whilst, since 25 γ produced about 53% increase in the actual weight of the prostate with seminal vesicles and 51% increase in the weight per unit of body weight, one rat unit of "whole male sexual activity" is contained in about 19 γ .

These two values for diol are close to the value of the capon unit (15–20 γ) given by the experiments of Tschopp, mentioned above, in which diol was injected for 6 days, but are about one-third of that given by Butenandt's method (2 days of injection, 50–60 γ per capon unit).

Direct experiments using these doses of 19 and 21 γ were not carried out, as the curves for the prostate (Figs. 1 and 2) show that a linear relationship between the dose and the percentage increase may be assumed for the range 0–50 γ .

It is clear from Tables II and III that the value of the rat unit is different for different doses injected in solutions of different concentration, the higher

Table III. *Influence of fat-soluble diol on prostate.*

The average weight, 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 injection.

No. of litter	Control rats	Rats injected with γ									
		25	50	100	200	350	25	50	100	200	350
		A. Actual weights, mg.					% increase				
1	52	—	—	110	139	—	—	—	112	167	—
2	54	—	101	—	—	—	—	87	—	—	—
3	52	—	99	—	158	—	—	90	—	204	—
4	54	—	—	122	—	—	—	—	126	—	—
5	53	—	—	—	136	—	—	—	—	156	—
6	51	—	—	108	—	161	—	—	112	—	216
7	59	—	—	—	—	165	—	—	—	—	180
8	52	78	—	—	—	—	50	—	—	—	—
9	52	72	—	—	—	—	39	—	—	—	—
10	51	79	—	—	—	—	53	—	—	—	—
Average	53	76	100	113	144	163	48	89	117	176	198
B. Calculated per 200 g. of body weight, mg.											
1	55	—	—	112	147	—	—	—	104	167	—
2	48	—	93	—	—	—	—	94	—	—	—
3	57	—	100	—	158	—	—	75	—	177	—
4	57	—	—	121	—	—	—	—	112	—	—
5	52	—	—	—	130	—	—	—	—	150	—
6	52	—	—	111	—	164	—	—	114	—	215
7	56	—	—	—	—	158	—	—	—	—	182
8	51	72	—	—	—	—	41	—	—	—	—
9	48	75	—	—	—	—	56	—	—	—	—
10	56	76	—	—	—	—	36	—	—	—	—
Average	53	74	97	115	145	161	44	85	110	165	199

doses producing effects, which are not directly proportional, the increase in the effect being less than the corresponding increase in the dose. This relation may be shown in the following way. If, instead of defining the rat unit as the minimum dose producing a 40% increase in the weight of the prostate (as required by our method of assay), it were defined as the proportion of any dose giving such an increase, then the number of rat units injected for the doses used would be as follows: 25 γ , 1.2 R.U.; 50 γ , 2.2 R.U.; 100 γ , 2.9 R.U.; 200 γ , 4.4 R.U.; 350 γ , 5 R.U.; or, on the basis of these calculations, one rat unit would appear to be contained in 21, 23, 34, 45 and 70 γ respectively.

In order to explain the different effects of different concentrations, it will be necessary to inject different doses with a solution of a fixed concentration.

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

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

Effect on the penis and preputial glands.

As can be seen from the figures of Tables IV and V the effect of diol on the penis was much more regular and comparatively greater than on the preputial

Table IV. *Influence of fat-soluble 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 injection.

No. of litter	Control rats	Rats injected with γ									
		25	50	100	200	350	25	50	100	200	350
		A. Actual weights, mg.					% increase				
1	86	—	—	156	170	—	—	—	81	98	—
2	93	—	145	—	—	—	—	56	—	—	—
3	84	—	140	—	191	—	—	67	—	127	—
4	81	—	—	158	—	—	—	—	95	—	—
5	95	—	—	—	170	—	—	—	—	79	—
6	90	—	—	158	—	180	—	—	76	—	100
7	93	—	—	—	—	189	—	—	—	—	103
8	79	115	—	—	—	—	46	—	—	—	—
9	81	104	—	—	—	—	28	—	—	—	—
10	81	107	—	—	—	—	32	—	—	—	—
Average	86	109	143	157	177	185	35	62	84	101	102
		B. Weights per 200 g. of body weight, mg.					% increase				
1	92	—	—	156	182	—	—	—	70	98	—
2	82	—	134	—	—	—	—	63	—	—	—
3	92	—	143	—	191	—	—	55	—	108	—
4	86	—	—	157	—	—	—	—	83	—	—
5	94	—	—	—	164	—	—	—	—	74	—
6	91	—	—	163	—	183	—	—	79	—	101
7	89	—	—	—	—	181	—	—	—	—	103
8	77	106	—	—	—	—	38	—	—	—	—
9	74	108	—	—	—	—	46	—	—	—	—
10	88	103	—	—	—	—	17	—	—	—	—
Average	87	106	139	155	179	182	34	59	77	83	102

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

Table V. *Effect of fat-soluble diol on prostate weighed with seminal vesicles and on preputial glands.*

Organs	Weights	Average % increase in weight of organs of rats injected with				
		25	50	100	200	350
Prostate with seminal vesicles	Actual	53	93	127	196	214
	Per unit body weight	51	91	118	186	235
Preputial glands	Actual	—	9	54	78	80
	Per unit body weight	—	8	49	67	80

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

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

glands, on which the smaller doses had no constant (25 γ) or considerable (50 γ) effect.

The curves obtained for the effect on the penis (Figs. 4 and 5) fit all the results obtained closely.

Effect on the fat deposition, the adrenals, the thyroid and the thymus.

It is difficult to attach a definite significance to the effect on these organs, since the changes were small and only occurred in some cases. Since, however, in the majority of rats the injections caused a decrease (per unit of body weight) in the weight of the adrenals (5–34 %) and of the thymus (3–26 %) it can be said that the effect was typical of a return towards normal in these organs.

Statistical description of the curves.

J. M. C. Scott, under supervision 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.

“The curves are approximately rectangular hyperbolas through the origin

$$y = \frac{bx}{x+a}.$$

These curves contain two adjustable constants a and b , but it is not easy to determine them by least squares. In practice therefore a curve

$$y = \frac{bx}{x+a} + \frac{cx^2}{(x+a)^2}$$

was constructed, where a was chosen beforehand and b and c were found by least squares. It can be proved that, if a is fairly near the correct value, the effect of the additional term is almost exactly equivalent to that of adjusting a , but the procedure is now the much simpler one of fitting a quadratic.”

DISCUSSION.

In Table VI, the data obtained in our previous experiments on androsterone [Korenchevsky and Dennison, 1935] are compared with the corresponding data of the effects of diol on the sexual organs.

Table VI. *Comparison of the effect of fat-soluble androsterone with that of fat-soluble diol.*

Organs	Weights	Average % increase per 100 γ for doses			
		I	II	III	IV
		200–900 γ androsterone	25–200 γ diol	200 γ androsterone	200 γ diol
Seminal vesicles	{ Actual	23.0	223	19	150
	{ Per unit of body weight	22.6	216	14	143
Prostate	{ Actual	24.7	144	24	88
	{ Per unit of body weight	23.7	135	20	83
Prostate with seminal vesicles	{ Actual	24.3	156	23	98
	{ Per unit of body weight	23.3	149	18	93
Penis	{ Actual	11.0	100	14	51
	{ Per unit of body weight	10.3	93	9	42
Preputial glands	{ Actual	18.0	37	29	39
	{ Per unit of body weight	18.0	33	23	34

In making the comparison the average percentage increase in the weight of the organs per 100 γ was chosen, using for both hormones those parts of the curves before the beginning of the pronounced flattening, *i.e.* for androsterone, the average percentage increase obtained after the injection of doses from 200 to 900 γ (column I) and for diol of doses from 25 to 200 γ (column II). In addition a comparison was made of the effects of the dose of 200 γ , which was used in both the androsterone and diol experiments (columns III and IV).

It is clear that the two substances differ considerably. The effect of diol on all the secondary sexual organs is much more powerful than that of androsterone. Thus the effect of diol on the seminal vesicles is about 8–10 times stronger than that of androsterone; on the prostate about 4–6 times and on the penis about 4 times with the higher doses and 9 times with the calculated average dose. The effect of diol on the preputial glands is about twice that of androsterone.

This difference is also shown by the comparison of the rat units of androsterone (170 γ) and diol (about 21 γ , *i.e.* 8 times greater).

Whilst the effect of androsterone on the seminal vesicles is about the same as or a little less than that on the prostate, in the case of diol the former glands are stimulated more than the latter.

A similar effect was also noted by Callow and Deanesly [1935], who, simultaneously with ourselves, carried out experiments using the principle of investigation advocated by us, *i.e.* judging the effect by changes in the weights of the organs fixed in Bouin's solution.

A comparison of the curves of increase in the weight of prostate in the androsterone assay [Korenchevsky and Dennison, 1935] and the diol assay shows a further difference—that, whilst the effect of androsterone is directly proportional to the dose within a comparatively large range of doses, this is not the case with diol.

In capons the effect of diol was found to be three times stronger than that of androsterone. In our present experiments the effect was found to be about eight times greater than that of androsterone. The data mentioned above indicate the importance of making the assay on mammals, in particular on rats, since it seems to be easier to differentiate between diol and androsterone on rats than on capons. For this purpose (*a*) the great difference in the values of the rat units and (*b*) the great difference in the effects on the seminal vesicles should be used.

SUMMARY.

1. Pure crystalline androsterone-diol prepared by Ruzicka was dissolved in olive oil and assayed by the authors' method on 49 rats belonging to 10 litters, the results being graphically represented by the curves for the prostate, seminal vesicles and penis.

2. One rat unit of "comb-growth activity" was found to be contained in less than 25 γ , in about 21 γ , whilst one rat unit of "whole male sexual activity" was contained in about 19 γ .

3. Thus, whilst on capons diol was found to be about three times stronger than androsterone, using castrated rats its activity was found to be about eight times that of androsterone.

4. In the assay of these two hormone preparations two features differentiate diol from androsterone: (1) the greater activity of diol as expressed by the rat unit and (2) its action in stimulating the growth of the seminal vesicles more than that of the prostate.

5. The effect of diol on the penis and on the preputial glands is also greater than that of androsterone, the reaction of the penis being more pronounced and regular than that of the preputial glands.

Grants from the Lister Institute and from the Medical Research Council have enabled us to carry out this work and to them our thanks are due. We are much indebted to Prof. E. S. Pearson, Mr J. M. C. Scott and the Department of Statistics of University College for the statistical investigation of the results obtained. We would also like to thank Messrs Ciba, Ltd., for supplying the material and Mr T. F. Smart for his assistance in obtaining this material.

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