# L. THE INFLUENCE OF THE FRESHNESS OF THE TESTES AND OF DESICCATION OF THE TESTICULAR TISSUE ON THE YIELD OF TESTICULAR HORMONE.

By VLADIMIR KORENCHEVSKY, ROSA SCHALIT and DORIS GRAETZ.

*From the Lister Institute, London.*

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The yield of testicular hormone varies for different batches of bulls' testes [Koch, 1930]. It is, therefore, important to make a study of the conditions which might influence the yield.

It has been shown that strong oxidation destroys the testicular hormone [Dodds *et al.*, 1930; Gallagher and Koch, 1930]. However, several stages in the extraction of the hormone from urine and testes were performed in the presence of air with satisfactory results. Moreover, the testicular hormone survives boiling with acids and with alkalis [Dodds *et al.*, 1930; Gallagher and Koch, 1930]. Dodds and his co-workers have also shown that the activity of the hormone is destroyed by pepsin and by trypsin. This fact suggested to us that the autolytic ferments present in the testicular tissue might also destroy the hormone. Oxidation, and more particularly the influence of autolytic ferments, might both be responsible for the lower yield of the hormone from testes kept for more or less prolonged periods before extraction. The testes supplied from the slaughter-house are of different degrees of freshness and it is a matter of technical difficulty to obtain the testes immediately the bulls have been slaughtered.

It was decided to study the effect of desiccation of the tissue, not only on the yield, but also from a second point of view, that of simplification of the method of extraction. The method of preparation of testicular hormone from bulls' testes which has been much used is that recommended by Koch and Gallagher [1929]. By this method the ground testicular tissue is first extracted with four times its weight of 96 % alcohol, which is afterwards removed by distillation. To save time and expense it was decided to study the influence of drying the tissue so as to obtain the material in such a condition as would permit of a direct benzene extraction of the hormone, without previous alcohol extraction and at the same time without decreasing the yield of hormone. Since the results of our experiments on "fresh" and "kept" testes seemed to

confirm our suggestion that the testicular autolytic ferments are harmful to the hormone, it was decided to destroy the testicular ferments by boiling the minced testicular tissue in water before drying it.

1. THE INFLUENCE OF THE FRESHNESS OF THE TESTICULAR TISSUE.

The greatest care was taken to obtain the testes at the slaughter-house immediately the bulls had been killed. The testes were put into containers surrounded with freezing mixture and brought to the Institute in a frozen condition.

The extracts "Fresh" and "Kept" were prepared in the following way.

The testes were minced, well mixed and divided into two equal parts. The one part, "Fresh," was used for immediate extraction, the second, "Kept," being left in a large open beaker at room temperature for about 20 hours. The "Kept" part was then used for the preparation of extract.

The method used for the extraction of the testicular hormone was that proposed by Koch and Gallagher [1929]. In order to save time the method was not followed to the final purification, being carried out only to the stage of extraction with 70 % alcohol from hexane solution, *i.e.* crude extracts only were prepared. At this stage a preparation is obtained which is readily absorbed from the subcutaneous tissue without producing indurations or a general toxic effect.

The extract obtained was emulsified at about $p_H$ 7·6–7·8, in 1 % gum acacia, to which had been added 0·3 % tricresol, distributed in ampoules and sterilised in the steamer 3 times for 30 minutes on 3 consecutive days.

Experiments were performed on 5 litters containing 19 castrated rats (Table I).

Table I. *Influence of the freshness of the testes on the activity of the testicular extract.*

| No. of extract injected | No. of litter | No. of rats in litter | Age of rats in days | No. of days after castration | Initial average weight of rats | Average weight of prostate + seminal vesicles in mg. per 200 g. of rat | | | Average weight of penis in mg. per 200 g. rat weight | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Control rats | Rats injected with "Fresh" extract | Rats injected with "Kept" extract | Control rats | Rats injected with "Fresh" extract | Rats injected with "Kept" extract |
| 89 | 1 | 5 | 116 | 74 | 254 | 65 | 405 | 146 | 154 | 218 | 183 |
| 89 | 2 | 2 | 94 | 27 | 260 | 48 | 154 | — | 75 | 124 | — |
| 89 | 3 | 2 | 280 | 129 | 399 | 199 | — | 291 | 224 | — | 258 |
| B.D.H. | 4 | 5 | 85 | 62 | 229 | 46 | 114 | 94 | 115 | — | — |
| B.D.H. | 5 | 5 | 93 | 26 | 208 | 184 | 260 | 206 | 136 | 178 | 151 |

Extract No. 89, prepared by us, was injected into the rats of litters Nos. 1 to 3, while extracts prepared by the British Drug Houses, designated in Table I by the letters "B.D.H.," were injected into the litters Nos. 4 and 5.

We have to thank the British Drug Houses and Dr F. H. Carr for very kindly supplying us with the extracts prepared for this experiment, and Dr A. T. Fuller and Dr S.W. F. Underhill for the actual preparation of the extracts.

The period of injection of our extracts was 7 days and of "B.D.H." extracts, 11 days. 10 mg. of the extract were injected subcutaneously into each rat twice a day.

The test used for the determination of the activity and strength of the extract injected, details of which are described in a previous paper [Korenchevsky, 1932], was the increase in weight of the prostate with seminal vesicles of injected castrated rats, as compared with the weight of those of uninjected castrated control rats. This was corroborated by the increase in weight of the penis of injected rats, although the response of the penis to the injections is not so sensitive as that of the prostate with seminal vesicles, as will be shown in a later paper. The results of the experiment are shown in Tables I and II.

Table II. *The yield of the testicular extracts.*

| Extracts | Yield in mg. from 1000 g. of testes | Extracts | Yield in mg. from 1000 g. of testes |
|---|---|---|---|
| No. 89 "Fresh" | 341 | 109 "K-G" | 561 |
| No. 89 "Kept" | 313 | 109 "Dry" | 285 |
| B.D.H. "Fresh" | 156 | 112 "K-G" | 996 |
| B.D.H. "Kept" | 89 | 112 "Dry" | 550 |
| | | 124 "K-G" | 923 |
| | | 124 "Dry" | 980 |

As can be seen from the Tables, the injection of both of the extracts prepared by us and of those prepared by the British Drug Houses gave the same results. In litter 1 (Table I) under the influence of "Fresh" extract the weight of prostate with seminal vesicles was increased by 523 % as compared with the uninjected control rats; whilst the "Kept" extract increased the weight of these organs by only 125 %. The respective figures for litters 2 and 3 were 221 % ("Fresh") and 46 % ("Kept"); for litter 4, 148 % and 94 %; and for litter 5, 41 % and 12 %.

The difference between the strengths of the "Fresh" and "Kept" extracts, prepared at the Lister Institute, appears to be much greater than the difference between the strengths of the respective extracts prepared by the British Drug Houses (see Table I). This discrepancy however greatly decreases if the yield is taken into consideration (Table II), the yield from 1 kg. of fresh testes being about double that obtained from kept testes in the "B.D.H." extracts. In our extracts the yield from fresh testes was only slightly greater than from kept material.

In litter 5, the "B.D.H." extracts were much less active than in litter 4. This was explained by the fact that the experiments on litter 5 were started 2 weeks later than those on litter 4; consequently the extract had been kept 2 weeks longer in the ampoules. This indicates that the extract loses much of its activity when kept as a weak emulson in 1 % gum acacia in the presence of air, which probably oxidises the hormone. Moreover we have been able to show the presence of oxidising ferments in the 1 % gum acacia solution used for emulsifying the hormone. These facts suggest that 1 % gum acacia is unsuitable if the hormone is to be preserved for long periods in emulsion form.

The results obtained show quite definitely that the yield of active testicular hormone is much greater from fresh testes than from testes kept for about 20 hours at room temperature.

### 2. The influence of drying the testicular tissue, previously boiled, on the yield of the hormone.

Bulls' testes were minced, well mixed and divided into two equal parts. One part was extracted by the method of Koch and Gallagher in the usual way to the same stage of purification as in the previous experiment, *i.e.* the final extract was prepared by extraction with 70 % alcohol from hexane solution.

The other part of the minced testes was thrown into about twice its volume of boiling water, acidified with 50 % acetic acid to $p_H$ 4·6–4·8. The mixture was boiled for 15 to 20 minutes, by which time the proteins had coagulated and also most of the ferments had been destroyed. The mixture was then filtered through a Büchner funnel and pressed out if necessary. The precipitate was then spread on sheets of glass, placed in the hot room at 37° and dried in a gentle current of air produced by an electric fan. A powerful current of air seems to be injurious to the activity of the preparation. If stirred occasionally, the tissue dries in about 5 to 12 hours, the speed of drying depending chiefly on the degree of removal of water from the boiled tissue by the press and the thinness of the layer on the glass.

A light brown, porous, brittle, dry mass was obtained, which was extracted twice with benzene (24 hours in all). The extraction proceeds very quickly because of the porous nature of the dried tissue, due to the coagulation of the proteins, which not only makes the extraction quicker and easier but also helps to hasten the process of drying.

The benzene extract was then treated by Koch and Gallagher's method to the same stage of purification as was the first portion of testicular tissue.

In what follows we shall designate the extract prepared by the Koch-Gallagher method as "K-G" extract and the extract prepared by the method as modified by us as "Dry" extract.

By the method detailed above we prepared, from three different batches of testes, three different pairs of the extracts "K-G" and "Dry." The strengths of these extracts were tested in the same way as in the previous experiments, and the results are summarised in Tables II and III.

10 mg. of the extracts were injected twice a day into the litters 5, 7 and 8, for 10, 9 and 12 days respectively.

As is shown by Table IV, in the first two batches prepared (extracts Nos. 109 and 112) the yield of "Dry" extract was less than that of "K-G" extract. The weights of prostate with seminal vesicles, and of penis, however, indicate that the strength of the same weight unit of the "Dry" extract was greater than that of the "K-G" extract (Table III) showing that, in these cases, a purer and more concentrated extract had been prepared by the "Dry"

method. From the third batch (extracts No. 124) the yields by the two methods were nearly the same (Table II), but the strengths were also nearly the same (Table III), being slightly greater in "Dry" extract than in "K-G" extract.

Table III. *Influence of desiccation of the testicular tissue on the activity of the testicular hormone.*

| No. of extract injected | No. of litter | No. of rats in litter | Age of rats in days | No. of days after castration | Initial average weight of rats | Average weight of prostate +seminal vesicles in mg. per 200 g. of rat | | | Average weight of penis in mg. per 200 g. rat weight | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Control rats | Rats injected with "K-G" extract | Rats injected with "Dry" extract | Control rats | Rats injected with "K-G" extract | Rats injected with "Dry" extract |
| 109 | 6 | 4 | 92 | 54 | 229 | 84 | 124 | 153 | 90 | 109 | 134 |
| 112 | 7 | 5 | 81 | 55 | 199 | 54 | 118 | 131 | 73 | 107 | 121 |
| 124 | 8 | 7 | 45 | 10 | 92 | 109 | 207 | 215 | 82 | 127 | 146 |

Table IV. *The influence of the testicular extracts 124 "K-G" and 124 "Dry" on the sexual organs of castrated rats of litter No. 8.*

| No. of rat | Group of rats | Weight of prostate +seminal vesicles in mg. | | Weight of penis in mg. | | Final weight of rats g. |
|---|---|---|---|---|---|---|
| | | Actual | Per 200 g. of rat | Actual | Per 200 g. of rat | |
| 1820 | Controls | 66 | 116 | 53 | 93 | 114 |
| 1821 | | 82 | 101 | 58 | 71 | 163 |
| | Average | 74 | 109 | 56 | 82 | 139 |
| 1822 | Test. ext. 124 "K-G" | 183 | 208 | 111 | 126 | 176 |
| 1823 | | 145 | 205 | 90 | 127 | 142 |
| | Average | 164 | 207 | 101 | 127 | 159 |
| 1824 | Test. ext. 124 "Dry" | 151 | 217 | 104 | 150 | 139 |
| 1825 | | 170 | 217 | 105 | 134 | 157 |
| 1826 | | 162 | 212 | 117 | 153 | 153 |
| | Average | 161 | 215 | 109 | 146 | 150 |

Differences in the completeness of coagulation of the proteins during the boiling process and in the degree of drying of the testicular tissue probably explain the difference in concentration of the testicular hormone in the "Dry" extracts Nos. 109, 112 and 124. The time of drying and the strength of the current of air produced by the electric fan might also have some influence. The yield of the final testicular preparations also varies for different batches (Table II). We rigorously followed the same procedure in every detail in preparing each pair of extracts from a batch of testes. The details however were slightly modified for different batches of testes. For example, in batches 89 and 109, 70 % alcohol by volume was used for the final extraction from hexane solution, whilst in batches 112 and 124, 70 % alcohol by weight was used. The extraction of the preparation from the hexane solution by 70 % alcohol was in some batches made only twice, in others more often. These results will be discussed elsewhere.

From the results obtained it is clear that it is possible to extract from the testicular tissue the same amount of testicular hormone by our modification of the method as by the original method. In order to show how the average figures of Tables I and III were obtained we give in Table IV the detailed figures for the comparison of the strengths of extracts No. 124 "K-G" and No. 124 "Dry."

## Conclusions.

1. The freshness of bulls' testes plays an important part in the yield of the hormone.

2. After boiling the testicular tissue in water acidified with acetic acid to $p_H$ 4·6–4·8, it is possible to dry it at 37° without appreciably decreasing the yield of the hormone.

3. Apart from oxidation, the autolytic ferments present in the testicular tissue are probably the most destructive agents of the testicular hormone in testes removed from the organism and kept at room temperature.

4. A simplification of the method of extraction of the hormone is possible by benzene extraction from testicular tissue, which has been first boiled and then dried at 37°.

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