LXIV. THE SEXUAL GLANDS AND METABOLISM.

VI. THE INFLUENCE OF WATER-SOLUBLE TESTICULAR AND PROSTATIC EXTRACTS FRACTIONATED AT VARIOUS ISOELECTRIC POINTS UPON THE NITROGEN METABOLISM OF RABBITS AND THE DEVELOPMENT OF THE GENITAL ORGANS OF RATS.

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HISTORICAL.

WE do not know of any work published on the subject which is mentioned above. An attempt to find the male sexual hormones among the lipoid fractions of the testis and prostate formed the subject of the previous paper [Korenchevsky, 1928]. The results which were obtained were not conclusive. The simultaneous injection of a particular fraction from the testis and from the prostate did sometimes increase the nitrogen metabolism, but never the growth and development of the male genital organs. The present paper deals with the results obtained by injecting various fractions of the water-soluble constituents of the sexual glands. In experiments on dogs, Korenchevsky and Bogoslovsky [1921] found that simultaneous injections of emulsions of prostate and testis produced a much greater effect upon the nitrogen metabolism than either separately. It was therefore suggested that the prostate has a function of inner secretion *and is in a synergetic relation* with the inner-secreting tissue of the testis. Later, Korenchevsky and Carr [1925, 1], in experiments on rabbits, showed that homologous prostatic emulsions alone increased the nitrogen metabolism and that the simultaneous injection of prostatic and testicular emulsions did not give a larger effect. This was ascribed to the fact that in the testis of the rabbit there are substances which depress nitrogen metabolism.

Hunt [1925] pointed out that in human patients the symptoms of deficient inner secretion of the testes are only re-established in a certain percentage of cases following successful transplantation of this gland. This indicates that the testicular hormone is not entirely responsible for the size of the sexual organs, their functional activity, the general mental and physical development, growth

of the hair, etc. As clinical cases of disturbance of the prostate often occur with the onset of impotence, Hunt combined testicular transplantation with treatment with a prostatic preparation. Oral administration of prostate tablets was without effect, but the effects of hypodermic injections of prostate extracts, prepared in the laboratories of Lilly and Co., have, he says, "been rather remarkable" and in each case a return of sexual function was stated to occur. These facts explain why, in the present investigation, the greater number of experiments was performed to study the influence of simultaneous injections of testicular and prostatic extracts.

EXPERIMENTAL.

In these experiments, for the purpose of testing the activity of the extracts, 51 rats and 18 rabbits were used. In selecting a method it seemed essential to choose one which would avoid the presence of insulin-like substances in the final products, for, as Korenchevsky and Carr [1925, 2] have shown, these may mask the effects of sexual hormones, since the latter seem to have weak and slow action as compared with that of insulin, which belongs to the group of strong and quickly acting hormones. In order to remove these substances the crude fraction was extracted with 80 % alcohol containing 2 % HCl.

Bull's or pig's testes and prostates were used for the isolation of the extracts, no difference being found between the extracts from the glands of these two species of animal.

The first stages of the method were similar to those of the method used by Collip [1925] for the extraction of the parathyroid hormone. The prostate or testis was minced in a Latapie's machine, mixed with an equal volume of 5 % HCl and left in the cold store for 2 days. The p_H , after diluting the mixture with an equal volume of distilled water, was then adjusted to 7.6–8.0 with weak NaOH solution. The material was then centrifuged, the liquid poured off and the residue re-extracted at p_H 7.6 and again centrifuged. The two supernatant fluids were united, made acid to Congo red and saturated with sodium chloride, the precipitate was filtered on a Büchner funnel and ground in a mortar with 80 % alcohol containing 2 % HCl, in order to extract the insulin-like substances. The material was centrifuged and re-extracted twice with 80 % neutral alcohol and again centrifuged. In some experiments sodium carbonate was used in place of hydrochloric acid [Dudley and Starling, 1924]. No difference was obtained in the results. The supernatant fluids were thrown away and the residue emulsified in distilled water and adjusted to p_H 7.6–7.8, at which point it is soluble. By adjusting the solution to p_H 6.0, a first precipitate was formed, which was obtained by centrifuging the material. A few preliminary experiments showed this to be inactive and it was therefore rejected. Large precipitates were obtained between p_H 5.4 and 4.8. These were obtained by centrifuging. The resulting fluid did not, except in a few cases, give any precipitate at p_H lower than 4.8. In a few cases a small precipitate was obtained at p_H 4.0. The usual process was therefore, after centrifuging off the precipitate

obtained at p_H 4.8 and if no precipitate was formed at p_H 4.0, to adjust to about p_H 3.0 with HCl and to precipitate the substances remaining in the liquid by saturating a second time with NaCl. In every case a very large precipitate was obtained and was filtered off on a Büchner funnel.

All the precipitates obtained as described above were dried in a vacuum over phosphorus pentoxide and when dry were ground in a mortar and kept in bottles, in desiccators, in the cold store.

The extracts of testis obtained at p_H 4.0 and 4.8 or by the second NaCl saturation will in this paper be called *AT* 40, *AT* 48 and *AT* 2*S* respectively. For the analogous prostate extracts the letter *P* will replace the letter *T*.

In those cases where the extracts were prepared in the same manner, but without extracting the insulin-like material with alcohol, the letter *A* will be omitted; for example, such a testis extract obtained at p_H 4.8 would be called "*T* 48."

A second method of fractionating was also tried. The crude extracts were converted into the hydrochlorides, using Dudley and Starling's insulin method [1924]. For this procedure extracts were used which had not been treated with alcohol. Only a part of the extract was found to form a hydrochloride soluble in acid alcohol. The soluble hydrochloride and the insoluble residue were both collected, dried and tested separately. Thus from all the extracts two fractions were obtained, a soluble hydrochloride and an insoluble residue. In these cases, to the abbreviations used above will be added the letters HCl; for example, *T* 48-HCl will be the hydrochloride of a testicular extract precipitated at p_H 4.8.

For the injections the dried extracts were ground in a mortar with a few cc. of water and adjusted to p_H 7.6–7.8. In about half of the experiments the extracts were subcutaneously injected in this form. Owing to the fact that the extracts, thus prepared, contained some insoluble matter, indurations were in some cases produced under the skin. These disappeared in about 1 or 2 weeks after the injections. In order to avoid this complication, for the rest of the experiments, the redissolved extracts were centrifuged in small sterile tubes and only the clear supernatant fluid was injected. In these no indurations or any other local reactions were noticed.

Analysis has shown that the crude extracts obtained at p_H 5.4 or 4.8 contained only about 28 % of organic soluble substances and those obtained after the second sodium chloride saturation about 96 % of soluble substances, of which about 17 % were inorganic salts (almost entirely NaCl).

The effect produced by a given weight of the extracts upon the metabolism was the same irrespective of whether the insoluble substances were, or were not, present in the injected extract. The amount of the extract injected was usually 0.03 g. per kg. per day, but in some experiments was 0.1 g. per kg. per day. The variation in the effect produced with varying doses was small.

The technique of the experiments in all other respects was exactly the same as that described by Korenchevsky [1925, 3] and Korenchevsky and Carr [1925, 1].

THE INFLUENCE OF THE EXTRACTS ON THE GROWTH AND DEVELOPMENT
OF THE GENITAL ORGANS OF CASTRATED RATS.

Experiments were performed on 51 rats, of which 17 were controls, the remaining being injected with the various extracts mentioned above. The single dose was from 25 to 50 mg. A total of twelve injections was made during the 30 days of the experiment. At the end of the experiment no difference could be detected between the penis, seminal vesicles and prostate of the injected rats and those of the control.

INFLUENCE OF THE EXTRACTS ON THE NITROGEN METABOLISM OF RABBITS.

Altogether 120 nitrogen metabolism experiments were performed on 18 rabbits, of which 10 were normal, 4 castrated, 3 parathyroidectomised and 1 thyroidectomised.

In order to shorten the record of the results, only those experiments are included in the tables in which the metabolic changes exceeded 5 % above or below normal during the period of injection, or the following period. For the same reason detailed figures of the metabolism experiments are omitted. In the tables only the percentage changes in the nitrogen excretion of the urine and the changes of the nitrogen balance in grams during the period of injection and one of the following control periods are stated. In this latter the figures are those of the period of greater change. In discussing the results obtained with each extract the number of experiments which have been omitted will be given.

1. *Testicular extracts.*

Extract T 48 precipitated from the alkaline solution at p_H 4.8. This extract was tested in the amount of 0.1 g. per kg. of rabbit. Three experiments were performed, in one of which no change was obtained and in two others (Nos. 9 and 10, Table I) a slight increase. There was no marked change in urinary flow. This fraction might be expected to contain insulin-like substances. In order to remove them a previous extraction of the testicular tissue with alcohol had been carried out, in the way described above.

Extract AT 48 precipitated at p_H 4.8 from an alkaline solution of the testicular substances which had previously been treated with alcohol. In all the experiments (Nos. 11 to 19, Table I), with the exception of No. 12, the nitrogen metabolism was markedly increased during the period of injection in No. 11 to 14 % and in No. 18 to 25 % above normal. In the control periods following the period of injection a decrease of nitrogen metabolism was found in all the experiments except No. 11. In some cases the amount of this decrease was higher than that of the increase of the metabolism (Nos. 12, 14, 16), which may indicate the incomplete removal of substances which depress nitrogen metabolism. In four experiments with the hydrochlorides of this extract no significant change in metabolism occurred.

Table I. *Influence of the extracts T 40, P 40, T 40 + P 40, AT 48, T 48 and P 48 upon the nitrogen metabolism.*

No. of exp.	Extract	Percentage change in nitrogen of urine		Change in nitrogen balance g.	
		Period of injection	Following control period	Period of injection	Following control period
1	T 40	+ 17.4	+ 0.9	- 0.30	- 0.07
2		+ 9.9	- 10.1	- 0.75	+ 0.78
3	P 40	- 0.7	+ 17.5	+ 0.06	- 1.48
4		+ 21.4	- 6.2	- 1.58	+ 0.46
5	T 40 + P 40	+ 0.6	- 5.0	- 0.23	+ 0.20
6		+ 28.6	- 6.7	- 2.62	+ 0.12
7		+ 8.5	- 7.4	- 0.67	+ 0.58
8		+ 1.1	+ 3.4	+ 0.41	+ 0.34
9	T 48	+ 7.6	- 10.6	- 0.40	+ 0.56
10		+ 1.7	+ 4.3	- 0.13	- 0.33
11	AT 48	+ 14.1	+ 12.5	—	—
12		+ 1.7	- 9.7	- 0.13	+ 0.90
13		+ 9.3	- 6.0	- 0.52	+ 0.34
14		+ 5.8	- 11.2	- 0.45	+ 0.87
15		+ 7.4	- 4.1	- 0.56	+ 0.31
16		+ 6.4	- 11.6	- 0.52	+ 0.95
17		+ 6.8	- 3.5	- 0.51	+ 0.26
18		+ 25.1	- 4.6	- 1.71	+ 0.33
19		+ 9.6	- 4.4	- 0.87	+ 0.40
20		+ 5.5	—	- 0.24	—
21	P 48	+ 13.1	- 17.2	- 0.19	+ 2.33
22		+ 5.3	—	- 0.21	—
23		+ 4.0	+ 5.5	- 0.11	- 0.23
24		+ 5.0	—	- 0.34	—
25		+ 5.0	- 3.4	- 0.32	+ 0.28
26		- 2.7	- 10.8	+ 0.32	+ 1.27

Note. In all the tables the figures with + (increase) or - (decrease) denote the changes from the normal, i.e. the control period previous to the injections.

Table II. *Influence of the extracts AT 48 + P 48 and T 48 + P 48 upon the nitrogen metabolism.*

No. of exp.	Extract	Percentage change in nitrogen of urine		Change in nitrogen balance g.	
		Period of injection	Following control period	Period of injection	Following control period
27	AT 48 + P 48	+ 19.9	+ 7.9	- 1.39	- 0.69
28		+ 10.7	+ 4.9	- 0.74	- 0.30
29		+ 8.2	—	- 0.63	—
30		- 0.1	- 8.3	- 1.01	+ 0.66
31		- 0.5	- 8.7	+ 0.04	+ 0.75
32		+ 20.0	—	- 1.25	—
33		+ 11.8	—	- 0.94	—
34		+ 0.1	+ 7.7	+ 0.01	+ 0.13
35		+ 2.1	- 7.6	—	—
36		+ 12.2	—	- 0.94	—
37		+ 5.2	+ 10.3	- 0.32	+ 0.07
38		- 0.9	- 11.5	+ 0.08	+ 1.05
39		+ 16.6	+ 14.6	- 0.82	- 0.69
40	T 48 + P 48	+ 12.1	+ 3.9	- 0.75	- 0.31
41		+ 3.1	- 19.8	- 0.57	+ 1.26

Extract T 40 precipitated at p_H 4.0 after the removal of fractions precipitated at p_H 4.8. Only two experiments were performed, the results obtained being similar to those obtained after the injection of AT 48 (see Nos. 1 and 2, Table I).

Extract AT 2S precipitated by saturation with NaCl after removal of the extracts precipitated at p_H 4.8 and p_H 4.0, also the hydrochloride of AT 2S. Twelve experiments were carried out with the extract AT 2S, in five of which no change in the nitrogen metabolism occurred. An increase was obtained in the remaining seven (Nos. 42 to 48, Table III). The degree and character of the increase were similar to those obtained after the injections of the two previous extracts. Changes in the urinary flow were irregular, an increase in eight experiments with a maximum of 51 % and a decrease in four experiments, with a diminution of 40 % in one instance, being obtained. An attempt to increase the activity and purity of this extract was made by converting the substances in the extract which would form a hydrochloride into this form. Altogether seven experiments were performed, but no improvement in the activity was obtained (Nos. 49 to 51, Table III); the remaining four showed even less change.

Table III. *Influence of the extracts T 2S, P 2S and their hydrochlorides upon the nitrogen metabolism.*

No. of exp.	Extract	Percentage change in nitrogen of urine		Change in nitrogen balance g.	
		Period of injection	Following control period	Period of injection	Following control period
42	AT 2S	+11.2	—	-0.75	—
43		+22.7	+ 7.1	-2.33	-0.54
44		+ 5.2	- 8.1	-0.40	+0.62
45		+11.7	+ 0.1	-0.52	+0.32
46		+ 9.2	- 8.1	-0.68	+0.65
47		+ 7.1	+ 2.9	-0.59	-0.24
48		+ 7.0	-10.2	-0.56	+0.81
49	T 2S-HCl	+13.9	+11.3	-1.64	-1.44
50		+ 3.1	-10.0	-0.27	+0.88
51		- 3.1	-11.0	+0.29	+2.06
52	P 2S	-18.6	- 5.4	+1.26	+0.41
53		+23.3	+21.6	-1.08	-0.99
54		+ 4.8	—	-0.03	—
55		+ 8.7	—	-0.09	—
56		+ 9.6	+14.4	-0.14	-0.41
57		+ 3.5	-11.7	-0.25	+0.82
58		+ 8.4	—	-0.65	—
59	P 2S-HCl	+13.9	+ 9.6	-1.31	-0.91
60		+19.1	+ 6.4	-1.78	-0.60
61		+ 9.2	—	-0.83	—
62		+12.4	—	-0.67	—

It was found during the process of converting the substances of the extract into the hydrochlorides that only a part of these were soluble in acid alcohol. The insoluble part was collected and dried separately but it was not found to

exert a significant influence on the nitrogen metabolism in the four experiments made. These experiments are omitted from the tables.

2. *Prostatic extracts.*

The fractions precipitated at p_H 4.8 and 4.0, and a third (P 2S), which was obtained by saturating the remaining solution with NaCl after the removal of the two fractions precipitated at their isoelectric points, and also the hydrochlorides of P 2S, were tested for physiological activity. Altogether 21 experiments were made, of which 20 are given in the tables (Nos. 3, 4, 20–26, Table I, and Nos. 52–62, Table III).

The influence of all these extracts upon the urinary flow was small and inconsistent. A decrease of nitrogen metabolism was observed in two only (Nos. 26, Table I, and 52, Table III). In the remaining experiments an increase during the period of injection or the following period was obtained, the maximum being 23.3 % above normal (No. 53, Table III). The hydrochlorides were no more efficient in their action (Nos. 59–62, Table III).

3. *The influence of simultaneous injection of testicular and prostatic extracts upon the nitrogen metabolism and urinary flow.*

The testicular extracts T 48, AT 48, or AT 48-HCl simultaneously injected with the prostatic extract P 48. Thirty-six experiments were made, in twenty-one of which no significant changes were obtained. The seven experiments with hydrochlorides were among the negative results, showing that the inactive substances of the precipitate at p_H 4.8 formed the hydrochloride. Four experiments were performed on thyroidectomised animals. The simultaneous injection of P 48 + AT 48 into animals without the thyroid produced no significant change in the nitrogen metabolism. The remaining experiments are given in Table II, from which it is clear that the simultaneous injection of these prostatic and testicular extracts produced an influence upon the nitrogen metabolism similar to that obtained by the injection of the separate extracts.

The changes in urinary flow were irregular, but sometimes large, 71 % above the normal in one experiment and 42 % below the normal in another. In twenty experiments out of thirty-six the change was not less than 20 % above or below normal. We have no definite explanation of these large and opposite effects of the injections.

The testicular extracts T 40, AT 2S or T 2S-HCl simultaneously injected with the corresponding prostatic extracts P 40, P 2S or P 2S-HCl respectively. Altogether fifty experiments were performed, five with the extracts precipitated at p_H 4.0, twenty with those precipitated by saturation with NaCl after the removal of the fractions precipitated at p_H 4.8 and p_H 4.0 and twenty-four on the hydrochlorides of T 2S and P 2S. In only twenty-eight experiments did the changes exceed the normal by 5 %. The figures of these experiments are given in Table I (Nos. 5–8) and in Table IV (Nos. 63–86).

Table IV. *Influence of the extracts AT 2S + P 2S and the hydrochlorides of T 2S + P 2S upon the nitrogen metabolism.*

No. of exp.	Extract	Percentage change in nitrogen of urine		Change in nitrogen balance g.	
		Period of injection	Following control period	Period of injection	Following control period
63	AT 2S + P 2S	+ 8.3	+ 1.4	+0.73	-0.12
64		+ 10.1	- 4.6	-0.59	+0.27
65		+ 14.2	+ 9.5	-1.57	-1.12
66		+ 29.2	+ 18.8	-3.20	-2.12
67		+ 12.4	—	-0.98	—
68		+ 13.6	+ 1.9	-0.94	-0.13
69		+ 11.9	—	-0.62	—
70		+ 6.5	- 3.6	-0.66	+0.19
71		+ 5.5	- 2.9	-0.17	+0.42
72		+ 10.7	- 3.7	-0.89	+0.31
73		+ 16.9	+ 22.2	-1.20	-1.57
74		+ 10.0	—	-0.82	—
75		+ 8.0	- 4.7	-1.12	+0.17
76		+ 12.7	-17.9	-1.03	+0.45
77		+ 7.2	-15.4	-0.69	+1.48
78		+ 6.7	-10.1	-0.70	+1.06
79		+ 7.7	- 9.4	+0.66	+0.81
80		+ 7.1	- 9.3	-0.71	+0.93
81		+ 7.9	- 3.4	-0.87	+0.53
82		+ 10.3	—	+1.23	—
83	T 2S-HCl +	- 3.2	-13.8	+0.34	+1.47
84	P 2S-HCl	+ 10.9	+ 2.8	-1.00	-0.26
85		+ 5.7	—	+0.16	—
86		+ 11.6	- 3.2	-1.28	+0.35

The effects on the nitrogen metabolism of the simultaneous injection of the extracts precipitated at p_H 4.0 and those precipitated by saturation with NaCl (2S) were similar. In both cases the increase in nitrogen excretion was about the same, the proportion of animals in which no effect was observed being 20 %.

Table V. *The average changes in the excretion of nitrogen in the urine during the period of injection of various extracts and during the following control period in the experiments given in Tables I to IV.*

Extracts	No. of experiments used for average	Percentage change in nitrogen of urine (average)	
		Period of injection	Following control period
T 48	2	+ 4.7	- 3.2
AT 48	9	+ 9.6	- 4.7
T 40	2	+ 13.7	- 4.6
AT 2S	7	+ 10.6	- 2.7
T 2S-HCl	3	+ 4.6	- 3.2
P 48	7	+ 5.0	- 6.5
P 40	2	+ 10.4	+ 5.7
P 2S	7	+ 5.7	+ 4.7
P 2S-HCl	4	+ 11.2	+ 8.0
T 48 + P 48	2	+ 7.6	- 8.0
AT 48 + P 48	13	+ 7.9	+ 1.0
T 40 + P 40	4	+ 9.7	- 3.9
AT 2S + P 2S	17	+ 11.3	- 1.3
T 2S-HCl + P 2S-HCl	7	+ 7.2	- 5.4

Hydrochlorides prepared from the extracts 2S, produced no changes in metabolism in about 71 % of the rabbits injected with this mixture. At the same time the average increase (Table V) of the nitrogen metabolism was less than was obtained with the original extract "2S," which had not been treated with HCl. Therefore the attempt to separate specific substances increasing nitrogen metabolism, by purifying the extracts by conversion into the hydrochlorides, was unsuccessful. The changes produced in the urinary flow were again inconsistent and ranged from 83 % above to 53 % below the normal, the figures in about 71 % of the experiments with 2S being below normal.

DISCUSSION.

In the foregoing paper by Korenchevsky [1928] it was shown that in the case of the lipoids extracted from testicular and prostatic tissue fractions could be isolated from each, which were little, if at all, active alone, but which, when one fraction from the testis and one from the prostate were injected together, sometimes increased the nitrogen metabolism of rabbits as much as 20 %. This corroborates the hypothesis of synergetic correlation between testicular and prostatic glands, suggested by Korenchevsky and Bogoslovsky in 1921 on experimental evidence and by Hunt in 1925 on the basis of clinical observations. The lipid fractions in Korenchevsky's experiments were, however, unable to prevent the atrophy of the penis, seminal vesicles and prostate of castrated animals, nor were they able to produce hypertrophy of the genital organs of normal rats. Until substances are isolated which are more consistent in their action on metabolism and can prevent the atrophy of the genital organs in castrated animals, an announcement of the discovery of the male sexual hormones cannot be justified. It is by no means necessary, however, that these two properties should be possessed by the one hormone.

An attempt was made to isolate the specific hormones as water-soluble protein or protein-like substances, these experiments being described in the present paper.

The results we obtained with these extracts were similar to those with the lipoids. At different p_H values, or by saturation with NaCl, fractions were obtained which stimulated nitrogen metabolism, but they were not able to stimulate the growth of the sexual organs of castrated or normal rats. As was the case with the lipoids, the action on metabolism of these isolated fractions was inconsistent, in some experiments being very slight or even absent or decreasing instead of increasing the metabolism.

As was the case with the lipid fractions, a large part of this inconsistency in the results is explained by the incidence of an insensibility very quickly developing in many animals after the first injection. In the following table (Table VI), a few examples are given confirming this conclusion. A mixture of the extracts A 48 or 2S was injected.

An increase in the dose does not generally make any difference. For example, rabbit 38 (Table VI) received at the second injection twice the dose

Table VI. *The influence of repeated injections.*

No. of rabbit	Percentage change in nitrogen of urine		No. of days between first and second injection
	First injection	Second injection	
37	+12.4	-5.7	1
38	+11.9	+6.5	1
34	+8.2	-4.2	1
1	+20.6	+3.5	1
1	+11.8	+0.1	1
19	+8.2	+0.2	1

given at the first injection. When the time between the following injections is more than 3 weeks, in some animals, the insensibility is less marked. For example, in the above table (Table VI) between the first and second experiment on rabbit 1 a period of 22 days of rest was given, although in both experiments the insensibility on the second day is very marked. However, even if all the experiments in which insensibility occurred are excluded, there remains a number of experiments in which only slight changes in the metabolism were obtained after the injections. Another possible explanation of the inconsistency of the results, suggested by Korenchevsky [1925, 1, p. 31; 2, p. 171; Korenchevsky and Carr, 1925, 1, p. 81] is as follows. The nitrogen metabolism is controlled in the living organism by different mechanisms, among which the inner secretion of the sexual glands plays an important, but not vitally necessary, part. The varying effects upon the metabolism obtained by the injection of different *impure* preparations of sexual glands may possibly be explained (1) by the presence in these preparations of non-specific principles (*e.g.* insulin-like substances); (2) by the varying degree of functional efficiency and therefore the varying reaction of the synergetic and antagonistic endocrine glands which are influenced by the injections. Coward and Burn [1927] noticed the remarkable fact that the individuality of the animals also plays a great part in their reaction to the injections of ovarian hormone. In their experiments on rats and mice the variation in the active unit of the oestrus-producing hormone was sometimes as great as 1000 %. From the point of view of this hypothesis all the preparations, lipoid [Korenchevsky, 1928], and water-soluble described in the present paper must be considered to be impure. Probably the removal of insulin-like substances caused the removal of some part, perhaps a considerable part, of the specific hormones, the avoidance of one difficulty producing another. It may be that some of the procedures used are destructive to the male sexual hormones. These hormones may be very unstable or only stored in the glands to a trifling extent. In any case in comparison with the ease with which powerful female sexual fractions can be isolated from the ovaries *the difficulty of preparing specific and powerful fractions from the testes must be emphasised.*

From private information we know that, besides the results mentioned in the historical review of the present paper, many others were unpublished, because the results obtained were entirely negative.

Doubt as to the existence of the specific male sexual hormones cannot be entertained, since the following facts are well established.

(1) Atrophy of the prostate, seminal vesicles and penis follows castration.

(2) This atrophy can be prevented by a transplantation of testis into the castrated animal.

Therefore the inner secretion of the male sexual hormone does exist and its specific hormones will no doubt be found sooner or later. A comparison of the activity of the different extracts, prepared by us, leads to the conclusion that the precipitates obtained at p_H 4.0 and by a final salting out, 2S, are the most active in stimulating the nitrogen metabolism (Table V). With the exception of prostate hydrochlorides, P 2S-HCl, all the hydrochlorides are definitely less active than the original crude extracts from which they were prepared. By the hydrochloride method probably those substances are isolated which depress the nitrogen metabolism, as is done by insulin-like substances. The exception of P 2S-HCl is probably due to the fact that the prostate does not contain a large amount of depressing substances, as has been shown previously [Korenchevsky and Carr, 1925, 2].

A comparison of the lipid extracts [Korenchevsky, 1928] with the water-soluble extracts P 40 and 2S shows the latter to be stronger in their action on the metabolism. A comparison of the respective tables in the two papers shows this fact definitely.

It is interesting *that the presence of substances stimulating nitrogen metabolism was found by us both in the lipid and in the water-soluble protein-like fractions of the male sexual glands*. The same fact was stated for the ovarian hormone, for Doisy and co-workers [1924] extracted the ovarian hormone by lipid solvents and Laqueur and his co-workers [1925, 1926, 1927], and Dickens, Dodds and Brinkworth [1927] by their method, finally obtained a powerful extract, in water, of the ovarian hormone. It seems that the male and female hormones are similar in this respect.

Our attempts to isolate active fractions we consider have failed in several respects. Yet the whole series of investigations of Korenchevsky and his co-workers [1914–1928] on sexual glands and metabolism demonstrates the influence of sexual glands in stimulating nitrogen metabolism and that this property is possessed by some of the fractions isolated.

At the present moment we are approaching the problem of isolation of the male sexual hormones from an entirely different angle, the results of which seem to be encouraging. These results we hope to publish shortly.

In conclusion, we summarise the main results of the experiments in this and our other papers of the series.

GENERAL SUMMARY.

(1) Up to the present time we have been unsuccessful in our attempts to prevent the atrophy of the genital organs of castrated animals by means of

feeding with fresh or dried testicular tissue, or by injecting fresh emulsions, or different extracts, prepared from *normal* testis.

(2) In the same experiments prostatic tissue separately, or ingested simultaneously with testicular tissue, also failed to stimulate the genital organs of castrated animals, as was similarly the case with injections of the prostatic extracts used separately or simultaneously with the corresponding testicular extracts.

(3) Taking into consideration the comparative ease with which powerful sexual extracts can be isolated from the ovaries, the great difficulty in preparing specific and active fractions from male sexual glands must be emphasised.

(4) Lipoid fractions prepared by us from testis, by the same method as that used for the preparation of an active ovarian extract, did not produce any significant influence upon the growth of the genital organs or the nitrogen metabolism.

(5) One of the other lipid fractions isolated, when injected simultaneously with the corresponding prostate fraction, stimulated the nitrogen metabolism to a moderate degree, thus supporting Korenchevsky's hypothesis of the presence in prostate of hormones which are synergetic to those of the testis.

(6) A method was described for the removal of depressing substances from the water-soluble extracts of bull's and boar's sexual glands and for the isolation of fractions which were able to raise the nitrogen metabolism of rabbits and rats to a moderate extent.

(7) The results indicate that of the water-soluble extracts of testis and prostate the more active are those precipitated at p_H 4.0, or obtained by a second salting out after the removal of the fractions previously precipitated.

(8) A simultaneous injection of these water-soluble testicular and prostatic extracts does not produce a stronger effect upon the nitrogen metabolism than is produced by the injection of one of the extracts.

(9) The effect of these extracts upon the urinary flow is sometimes very marked, but is on the whole inconsistent.

(10) There is no difference in the influence of these extracts upon normal, castrated or parathyroidectomised animals. Thyroidectomised animals have never shown any significant rise of nitrogen metabolism after these injections.

(11) The water-soluble extracts seem to be more active than the extracts soluble in lipid solvents which we prepared. It is noteworthy that the ovarian hormone has been isolated both as lipid and water-soluble substances.

(12) Neither the water-soluble, nor the lipid fractions, which have been isolated, can be regarded as perfect preparations, since their action is often inconsistent. This is probably due to the presence in them of impurities, or to destruction of the active principles, or their loss with the rejected material, during the process of purification.

(13) Insensibility to a second injection of the extracts was noticed in several experiments. It can develop 24 hours after the first injection of water-soluble extracts. This may also explain some of the cases of inconsistent action of the extracts.

(14) Besides these two suggested causes of the inconsistent influence of the extracts isolated, the varying degree of functional state and therefore the varying reaction of the synergetic and antagonistic endocrine glands to the injections are also responsible for the cases of weak, or variable, effect of the extracts. Other investigators have also observed inconsistency in the action of ovarian hormone, possibly due to the same causes which have been already mentioned. Therefore this inconsistency appears to be common to both the male and female sexual hormones.

(15) The inner secretion of the sexual glands stimulates the whole organism. Therefore, theoretically the inner secretion would be expected to have a stimulating effect on metabolism. This suggestion is supported by the whole series of experiments of Korenchevsky and his co-workers [1914–1928] and by many other investigations, which have been discussed in the historical reviews of our publications.

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