LXIII. THE SEXUAL GLANDS AND METABOLISM.

V. THE INFLUENCE OF LIPOID EXTRACTS OF THE TESTES AND PROSTATE ON THE NITROGEN METABOLISM OF RABBITS AND ON THE DEVELOPMENT OF THE GENITAL ORGANS OF RATS.

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HISTORICAL.

Testes.

ISCOVESCO was the first investigator to study the lipoids of the gonads [1912, 1, 2]. He [1907–1914] extracted the lipoids of several tissues and glands, including the testis, and came to the conclusion that all the organs which he had investigated contained at least one specific lipoid which, when introduced into the organism, caused an increase in the function of the organ from which the lipoid had been isolated. Iscovesco therefore called these lipoids *homostimulines*. From a study of the action of various fractions of the lipoids he concluded that some of them are physiologically indifferent, others antagonistic to homostimulines. These he called *heterostimulines*.

Briefly, Iscovesco's method of isolation of the lipoids is as follows. To the minced tissue about three volumes of 95 % alcohol are added. After a few hours the alcoholic extract is filtered off and from this group I (alcohol-soluble) lipoids are isolated. The remaining tissue is desiccated, powdered and successively extracted with ether (group II, ether-soluble lipoids), with acetone (group III, acetone-soluble lipoids), with chloroform (group IV, chloroform-soluble lipoids) and again with alcohol (group V, alcohol-soluble lipoids). Each of these groups is further divided, as, for example, group II. The extract left after evaporation of the ether is mixed with light petroleum, by means of which it is divided into an insoluble portion, *A*, and a soluble portion, *B*. The *B* portion is evaporated and again dissolved in ethyl ether. If there is any precipitate, it is removed by centrifuging. The clear liquid is mixed with two volumes of acetone and left in the cold room for 24 hours. A precipitate, *C*, results, which is gathered on a filter-paper. The filtrate is concentrated and again left in the cold room and a second precipitate, *D*, is collected by filtration. The filtrate is evaporated and the residue extracted with boiling alcohol in

which it is completely soluble. After cooling for 24 hours it is separated into a precipitate, *E*, and the material in solution, *F*. Nearly all the cholesterol is collected in *E*. In their turn, each of these *A-F* fractions is treated in succession with light petroleum, ethyl ether, cold and boiling alcohol. In this way fractions *a, b, c* etc. are made from each of the previous fractions *A, B, C, D, E* and *F*.

Testicular specific homostimulines were found in the second ether-soluble group, fraction II *Fb* and in the fifth alcohol-soluble group, fraction V *Dc*. Iscovesco emphasises the fact that the testes contain several non-specific heterostimuline lipoids, some of which have a strong antagonistic influence and some of which are physiologically inactive.

In 2 or 3 months 34 to 45 subcutaneous injections of 0.01 g. per kg. of fractions II *Fb* or V *Dc*, dissolved in olive oil, produced hypertrophy of the testes in rabbits, the testes becoming two or three times heavier than those of the uninjected control animals. The general growth and condition of the rabbit also improved. The total amount injected was from 0.5 to 0.6 g. of the lipid fraction. Doses, which were too large (about 0.03 g.), of fraction II *E* produced in some animals paralysis of the hind legs. It is to be regretted that the number of experiments performed and some important details were omitted from this paper.

Hoskins [1927], in collaboration with Brown and Lee, was unable to obtain any influence on the voluntary activity of male rats with lipid extracts prepared by Allen and Doisy's method. Feller [1913, 1920] found among the lipoids extracted with alcohol and light petroleum from placenta, foetal membranes, corpus luteum, ovary and testicles, a fraction which produced hypertrophy of the uterus. The lipid extracted from testes produced atrophy of the spermatogenic cells of the testes, but a slight hypertrophy of the interstitial cells, with an increase of their lipid granules. No change was observed in the prostate or seminal vesicles. The corresponding lipid fractions from the ovary and from the placenta produced the same change in testicular cells. This interesting fact was corroborated by Herrmann and Stein [1916]. They injected a lipid fraction of placenta, or of corpus luteum, which they had prepared, into male rabbits. Atrophy of the testes, prostate and seminal vesicles resulted. Histologically the spermatogenic cells of the gonads were found to be atrophied, while the interstitial tissue seemed to have increased slightly. In another experiment they removed one testicle from a rabbit. The remaining testicle, after the lipid injections, was found, on comparing it with the testicle which had been removed, to have developed the same changes as those mentioned above.

Glimm and Wadeln [1926], using a lipid fraction extracted from placenta, and Laqueur, Hart and De Young [1925, 1926, 1927], by injecting their water-soluble oestrus hormone (menoform), obtained from follicular fluid, inhibited the growth of the genital organs of the male animals.

Schroeder [1920] produced hypertrophy of the *utriculus masculinus* in the male rabbit by injecting a lipid extract which he had prepared from placenta.

Prostate.

We do not know of any investigations in which lipid extracts of the prostate have been shown to have any physiological action.

EXPERIMENTAL.

In order to discover whether the various fractions isolated by us contained specific sexual hormones, two kinds of tests have been applied: first, the influence of isolated fractions on the growth of the genital organs of normal and castrated animals, and second, the influence on the nitrogen metabolism which is partly controlled, as our previous investigations have shown, by the inner secretion of the sexual glands.

Technique.

For the preparation of lipid extracts bulls' testes or prostate were used, and were brought from the slaughterhouse just after the animals had been killed. After the removal of fat and membranes, the glands were minced in a special Latapie's grinding machine. The method of fractionation was based on that of Doisy *et al.* [1924], successfully applied by them to the ovary. This method and various modifications of it were used in the attempt to isolate a testicular hormone. In the following account the former will be called the American method. In the American method the extracts are heated. As the degree of thermostability of testicular lipoids is not known, it seemed reasonable to modify it so as to avoid the use of high temperatures. This method will be called the ether method.

In the American method, after the first two alcoholic extractions of the tissue, the residue is thrown away. Since preliminary experiments showed that this residue still contained lipoids, it was decided to extract these. It was also considered advisable to eliminate from the fractions the insulin-like substances which are present in large amount in several tissues and particularly in testes. As has been shown previously [Korenchevsky and Carr, 1925, 1], these substances mask the effect given by the sexual hormones. To remove them, the final processes were carried out with anhydrous alcohol, ether and acetone, in which insulin is insoluble.

The practical details of the following method, which is described below, were carried out with the kind assistance of Mr F. H. Carr, to whom and to Messrs British Drug Houses, Ltd., my thanks are due. To test the technique of the method of preparation of sexual lipoids, Mr Carr was so kind as to have prepared in the Laboratory of Messrs British Drug Houses, Ltd., one batch of the extracts. The chemical and physiological properties were investigated at the Lister Institute and were found to be identical with those of the batches, prepared by us at the Lister Institute.

The method, as used, was the following. To the minced tissue of the glands an equal volume of 96 % alcohol was added, and after standing for 1 to 2 hours at room temperature the mixture was left in the cold room overnight. The next

day the mixture was filtered on a Büchner funnel and the residue pressed in a hydraulic press. The fluid so pressed out was added to the filtrate. The residue was re-ground in a mortar with an approximately equal volume of absolute alcohol, filtered, and the filtrate added to those already obtained. The mixture of all the filtrates above was called *A*.

The residue from the filtrate *A* was ground once more with absolute alcohol and filtered. The same procedure was repeated twice more and the filtrates combined. The residue was then extracted in a Soxhlet apparatus with ether and afterwards with boiling acetone. After evaporating off the ether and acetone in a vacuum the second alcoholic filtrates were added to the residue giving the second mixture *B*.

The mixture *A* was distilled to dryness in a vacuum and the residue divided into two equal parts, one part being used for the ether method (lipoid fraction No. 1) and the other for the American method (lipoid fraction No. 2). The mixture *B* gave in the same way lipoid fractions No. 3 (ether method) and No. 4 (American method).

In the following account all the testicular extracts are denoted by the letter *T* and the prostate extracts by the letter *P*. For example, P_3 denotes the lipoid fraction No. 3 prepared from prostate. The American method was used as it was described by Doisy *et al.* [1924]. The ether method was carried out as follows. The dry residue from the mixture *A* or *B* was washed into large centrifuge tubes with about 100 cc. of anhydrous ether, in which it is almost completely soluble. It was then left for a few hours or until the next morning in the cold room. The mixture was centrifuged while still cold and the clear ether extract poured off, the precipitate being thrown away. The fluid was distilled to dryness in a vacuum and the residue so obtained kept in nitrogen in the dark in the cold store. The lipoid fractions No. 2 and No. 4 were kept in the same way in order to prevent oxidation and decomposition by light and air. The injections were prepared by emulsifying, in a mortar, the weighed amount of the lipoid extract with saline solution, made slightly alkaline with NaHCO_3 . Good emulsions were obtained in this way.

The nitrogen metabolism experiments were performed by the method described elsewhere [Korenchevsky and Carr, 1925, 1, 2].

The influence of injections of lipoid extracts on the growth of the genital organs and thymus of normal and castrated rats.

One hundred and two rats (belonging to 16 litters), of which 92 were castrated and 10 normal, were used in the experiments. Each litter was divided into a control group and an injection group, the injections being made a few days after the operation had been performed. Altogether 17 injections of from 0.02 g. to 0.05 g. of the lipoid extracts were made during 45 days. The total number of control rats was 34, the number injected with T_1 6, with T_2 8, with T_3 8, with T_4 5, with P_1 3, with P_2 6, with P_3 8, with P_4 3, with a mixture of $T_1 + P_1$ 3, with $T_2 + P_2$ 3, with $T_3 + P_3$ 11, and with $T_4 + P_4$ 4. At the end

of the experiment the rats were killed and the weights of the prostate, the seminal vesicles and the thymus and the measurements, in mm., of the penis and the seminal vesicles of the injected animals compared with those of the control animals. It is well known that the penis, prostate and seminal vesicles atrophy after castration, whilst the thymus persists for an unusually long time or may even hypertrophy. No difference was observed between the injected and the control rats in either normal or castrated litters.

The influence of lipid extracts on the nitrogen metabolism of rabbits.

Testicular extracts. Twenty-eight experiments were performed on 11 rabbits of which 6 were normal, 2 castrated and 3 thyroidectomised. The amount of each extract injected each day, per kg. of rabbit weight, varied from 0.03 to 0.2 g. When a mixture of testicular and prostatic extracts was injected simultaneously the amounts of each of the two extracts were equal to half of that which was injected singly, for the purpose of comparing the results. In the control periods saline solution was injected. In the tables the total amount, per kg. of rabbit weight, injected is shown. In most cases the experiment was divided into four periods: a first control period, of 2 to 3 days before the injections, the period of injection of from 2 to 3 days, followed by two or three control periods, each of from 2 to 3 days. Usually at least 1 month divided two experiments on any one rabbit, but in some experiments, when the nitrogen metabolism had returned to normal, a second injection was made about a week after the first. In order that the number of tables may be decreased we give one in detail, as an example, and four tables giving the general results showing the changes in the urinary flow, in the excretion of the nitrogen of the urine and in the nitrogen balance.

Table I. *The influence of simultaneous injection of the extracts of testes T_3 and of prostate P_3 upon the nitrogen metabolism and urinary flow of the normal rabbit No. 7.*

No. of period	Length of period hrs.	Weight of rabbit g.	Total nitrogen intake g.	Volume of urine excreted cc.	Nitrogen in urine g.	Nitrogen in faeces g.	Total nitrogen output g.	Balance of nitrogen g.	Remarks
I	48	2910	10.42	354	6.65	2.86	9.51	+0.91	Injected saline
II	48	2860	10.42	616	8.30	2.86	11.16	-0.74	Inj. 0.5 g. $T_3 + P_3$ (0.25 g. of each)
III	48	2850	10.42	326	6.87	2.86	9.73	+0.69	Injected saline
IV	72	2830	10.42	233	6.50	2.86	9.36	+1.06	"
V	48	2840	10.42	238	6.35	2.86	9.21	+1.21	"

Subcutaneous injections of the testicular lipid extracts T_1 , T_2 , T_3 and T_4 (Table II) produced only small changes of varying character in the excretion of urinary nitrogen and in the balance of nitrogen. For example, the greatest increase of excreted nitrogen in the urine was 5.7 % above normal, while the greatest decrease was 6.7 %. In the control period following the injection

period the change persisted, except in one experiment, in a less marked degree. The dose injected did not influence the results obtained.

Although in some experiments the change of urinary flow was considerable, at the same time this was inconsistent. For example, in two experiments the change was 43.3 % and 25.3 % above normal and in another two experiments 14.4 % and 16.3 % below normal (Table II).

Table II. *The influence of subcutaneous injections of lipid testicular extracts Nos. 1, 2, 3 and 4 upon the nitrogen metabolism and urinary flow of rabbits.*

No. and description of rabbit	Total amount in g. per kg. and kind of extract injected	Percentage change in excretion of urine		Percentage change in nitrogen of urine		Change in nitrogen balance	
		Period of injection	Following control period	Period of injection	Following control period	Period of injection	Following control period
		%	%	%	%	g.	g.
1. Castrated	0.27 T_1	-16.3	-24.0	-3.4	-7.1	+0.30	+0.63
1. Castrated	0.17 T_2	+1.3	+6.3	+1.9	+6.9	-0.14	-0.52
6. Castrated	0.18 T_2	+25.3	-25.7	-1.5	-3.9	+0.15	+0.38
28. Normal	0.21 T_2	-1.6	-16.7	-0.1	-3.4	+1.04	+1.05
25. Normal	0.16 T_3	+43.3	+0.8	+5.7	+1.0	-0.47	-0.08
2. Thyroidect. and castrated	0.45 T_3	-1.5	-19.5	-2.3	-5.3	+0.19	+0.43
8. Thyroidect. and castrated	0.21 T_4	-14.4	-29.9	-6.7	-8.6	+0.26	+0.43

Note. In Tables II to IV the figures marked + (increase) or - (decrease) denote the changes from the normal, i.e. the control period previous to the injections.

Table III. *The influence of subcutaneous injections of lipid prostatic extracts Nos. 2 and 3 upon the nitrogen metabolism and urinary flow of rabbits.*

No. and description of rabbit	Total amount in g. per kg. and kind of extract injected	Percentage change in excretion of urine		Percentage change in nitrogen of urine		Change in nitrogen balance	
		Period of injection	Following control period	Period of injection	Following control period	Period of injection	Following control period
		%	%	%	%	g.	g.
28. Normal	0.09 P_2	+1.9	-4.0	+4.1	-2.5	-0.40	+0.24
6. Castrated	0.14 P_2	-2.2	-9.3	+7.5	-2.2	-0.68	+0.20
19. Normal	0.10 P_3	+7.2	-14.0	+7.9	+5.0	-0.73	-0.46
25. Normal	0.14 P_3	+1.4	-13.6	+0.9	-9.7	-0.09	+0.96
19. Normal	0.20 P_3	+16.0	-8.7	+8.2	+3.2	-0.59	-0.23
15. Normal	0.16 P_3	-9.8	-12.1	+4.6	+10.7	-0.36	-0.98
6. Castrated	0.14 P_3	+1.3	+15.4	-2.1	-4.9	+0.20	+0.48

Prostatic extracts. Of these, only two fractions, P_2 and P_3 , were investigated as short preliminary experiments showed that the fractions P_1 and P_4 had, within the limits of physiological fluctuations, only a small and uncertain influence.

Of seven experiments (Table III) a slight increase of urinary nitrogen and a negative nitrogen balance was noticed in five experiments, while in two experiments there was practically no change. In the control periods following the period of injection the same change of the nitrogen metabolism was still

present or became reversed. The greatest increase of nitrogen excretion in urine was 8.2 % in the injection period and 10.7 % in the control period. There was no appreciable difference in the reaction of normal and castrated animals.

Changes in the urinary flow were inconsistent and not very marked.

The simultaneous injection of testicular and prostatic extracts.

No significant changes were produced when fractions Nos. 1, 2 or 4 were used (Table IV). In half of the experiments fraction No. 3 moderately increased the nitrogen metabolism up to a maximum of 19.9 % above normal. However, in the other half of the experiments on the injection of the same fraction the metabolism was slightly decreased or practically unchanged.

Table IV. *The influence of simultaneous subcutaneous injections of lipid testicular and prostatic extracts Nos. 1, 2, 3 and 4 upon the nitrogen metabolism and urinary flow of rabbits.*

No. and description of rabbit	Total amount in g. per kg. and kind of extract injected	Percentage change in excretion of urine		Percentage change in nitrogen of urine		Change in nitrogen balance	
		Follow-		Follow-		Follow-	
		Period of injection %	ing control period %	Period of injection %	ing control period %	Period of injection g.	ing control period g.
15. Normal	0.13 $T_1 + 0.16 P_1$	+ 6.6	- 10.4	- 4.2	- 7.6	+ 0.43	+ 0.78
25. Normal	0.16 $T_2 + 0.19 P_2$	+ 13.6	+ 26.2	+ 6.8	+ 5.1	- 0.67	- 0.50
19. Normal	0.08 $T_2 + 0.08 P_3$	+ 0.5	+ 2.8	- 0.7	+ 3.2	+ 0.06	- 0.29
28. Normal	0.20 $T_3 + 0.08 P_3$	+ 15.3	- 23.1	+ 8.7	+ 9.5	- 0.77	- 0.95
28. Normal	0.05 $T_3 + 0.10 P_3$	- 14.1	- 27.8	+ 15.0	+ 9.6	- 1.20	- 0.77
25. Normal	0.03 $T_3 + 0.05 P_3$	- 4.3	- 30.4	- 6.0	+ 15.1	+ 0.59	- 1.49
25. Normal	0.04 $T_3 + 0.10 P_3$	+ 6.7	+ 24.9	+ 10.0	+ 17.5	- 0.66	- 1.15
7. Normal	0.09 $T_3 + 0.09 P_3$	+ 74.0	- 34.2	+ 19.9	- 4.5	- 1.65	+ 0.30
19. Normal	0.14 $T_3 + 0.14 P_3$	- 6.1	- 6.7	- 2.3	- 8.7	+ 0.21	+ 0.81
15. Normal	0.14 $T_3 + 0.14 P_3$	- 9.6	- 15.0	- 3.0	- 9.7	+ 0.27	+ 0.88
20. Normal	0.14 $T_3 + 0.14 P_3$	- 7.0	- 18.4	- 11.1	- 12.9	+ 0.81	+ 0.94
1. Castrated	0.14 $T_3 + 0.14 P_3$	- 4.6	- 9.7	+ 2.8	- 6.5	- 0.22	+ 0.05
2. Thyroidect. and castrated	0.14 $T_3 + 0.14 P_3$	+ 0.0	+ 8.3	- 5.5	- 25.4	+ 0.34	+ 1.55
9. Thyroidect.	0.10 $T_4 + 0.09 P_4$	+ 28.5	+ 25.6	+ 1.3	- 5.5	- 0.11	+ 0.46

To explain these divergent results the following suggestion may be considered. Rabbits 1, 15, 19 and 20, about 1 month before the present investigation, were used in an investigation on the influence of another extract of sexual glands and they failed to show an increase in nitrogen metabolism after the injection of $T_3 + P_3$.

Rabbits 7, 25 and 28, injected with the same mixture, were either new rabbits, used for this investigation only, or at least 2 months had elapsed since the previous experiment with them. In these nitrogen metabolism increased in every case. The number of these rabbits is small for definite conclusions. However, the difference in the reaction to the injections of both these groups of rabbits may indicate the appearance in them after about 1 month of an insensibility to a second injection. This insensibility may later disappear.

However, taking into consideration the comparatively small number of the experiments, the divergent results mentioned obtained after the injection of $T_3 + P_3$ may be explained by the *individual* reaction of the various animals. Previously [Korenchevsky, 1925, 1, 2] it has been shown that castration or injection of emulsions of sexual glands gives results varying in degree. The explanations given (see pp. 169–171) might be equally applied in this case. The changes in nitrogen metabolism of rabbits 25 and 28 are especially notable as regards the respective activity of the different fractions (injected singly or as a mixture) of testes and prostate (Tables II, III, IV). The results obtained with *thyroidectomised* rabbits must be considered quite separately. In our previous experiments [Korenchevsky and Carr, 1925, 2] we found that in thyroidectomised animals the injections of the emulsions of the sexual glands caused no change or a decrease of the nitrogen metabolism. This is also true in the case of all the extracts used in the present investigation (see rabbits 2 and 8, Table II, and rabbits 2 and 9, Table IV). The *urinary flow* after the injection of a mixture of the different fractions was in some experiments much changed, as for example in the experiment on rabbit No. 7 (Tables I and II), when the increase was 74 % above normal. The divergence, however, in the volume of urine excreted in the other experiments was so great that it is impossible to draw any definite conclusions. The individuality of the animal seems to play an important part in the changes of urinary flow. The importance of individuality was strikingly emphasised in a recently published investigation on the ovarian hormone [Coward and Burn, 1927].

SUMMARY.

1. A method is described for the isolation of lipid fractions of the prostate and testis, which were effective in raising the nitrogen metabolism.
2. Whilst these fractions (T_3 and P_3) were little, if at all, active when injected separately, when injected simultaneously they increased the nitrogen metabolism of some rabbits, sometimes as much as 20 %.
3. This increase in the nitrogen metabolism was obtained in only half of the experiments. A possible explanation is suggested.
4. Although the changes of urinary flow were in some experiments considerable, they were of so inconsistent a nature that no conclusions are drawn.
5. None of the fractions isolated from the prostate or testes, injected singly or mixed in the doses given, is able to prevent the atrophy of the penis, seminal vesicles and prostate of castrated rats, or is able to produce hypertrophy of the genital organs of normal rats.

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