BRITISH MEDICAL JOURNAL

LONDON SATURDAY APRIL 20 1940

KIDNEYS AND SEX HORMONES

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When in the course of previous investigations we began recording the weight of the organs of rats, we found, unexpectedly, that not only the weights of the endocrines but those of other important organs (liver, kidneys, and heart) are also influenced by sex hormones. The object of this paper is to explain the nature of the changes in the weights of the kidneys and thus to show whether any of the sex hormones are physiologically stimulating nephrotrophic factors, and, if so, which; and which of them produce harmful pathological effects.

Historical

EFFECT OF CASTRATION AND MALE HORMONES

The first observations on kidneys were made during experiments of comparatively short duration (twenty-one days), when it was found that purified extracts of male sex hormones from urine increased the weight of the kidneys (both actual and per unit of body weight) in normal and castrated rats (Korenchevsky, Dennison, and Kohn-Speyer, 1933a, 1933b). On the other hand it was found that castration was followed by a decrease in the weight of these organs (Korenchevsky and Dennison, 1934a, 1935a).

These conclusions (decrease after castration and enlargement after injections of male hormones) were confirmed repeatedly in subsequent publications on the effect of different pure crystalline compounds: for androsterone and androstanediol in male and female gonadectomized rats (Korenchevsky, Dennison, and Levy Simpson, 1935; Korenchevsky, Hall, and Ross, 1939; Korenchevsky, Hall, and Burbank, 1939); for testosterone propionate and dipropionate in gonadectomized and normal males and females (Korenchevsky, Dennison, and Eldridge, 1937b; Korenchevsky, Dennison, and Hall, 1937a, 1937b; Korenchevsky, Hall, Burbank, and Ross, 1939; Korenchevsky, Hall, and Ross, 1939; Korenchevsky and Hall, 1939; Korenchevsky, Hall, and Burbank, 1939).

Pure testosterone had a pronounced effect only in some experiments on gonadectomized males, but not, with the

doses used, in gonadectomized females or in normal animals of either sex (Korenchevsky, Dennison, and Brovsin, 1936; Korenchevsky, Dennison, and Hall, 1937b; Korenchevsky and Hall, 1939; Korenchevsky, Hall, and Ross, 1939).

The weak male hormones, transdehydroandrosterone, Δ^4 -androstenedione, and Δ^5 -androstenediol, apparently have no definite hypertrophying effect on the kidneys (Korenchevsky and Dennison, 1936; Korenchevsky, Dennison, and Eldridge, 1937a; Korenchevsky, Hall, and Ross, 1939; Korenchevsky, Hall, and Burbank, 1939; Korenchevsky and Hall, 1939). Nath (1939) found that artosterone, a hormone possessing male properties and isolated from the Indian fruit *Artocarpus integrifolium*, also increases the weight of the kidneys.

EFFECT OF OVARIECTOMY AND OESTROGENS

Although the kidneys of male rats were found to be smaller after castration, no definite or constant change was found in the actual weights of the kidneys of female rats after ovariectomy (see those papers already mentioned in which experiments were made on both sexes). Since the body weight of spayed rats is greater than that of the controls, the weight of the kidneys per unit of body weight is slightly smaller in spayed animals than in controls. This may suggest some similarity between the effects of gonadectomy in both sexes.

Oestrogenic hormones do not appear to have a constant effect on the weights of the kidneys in either sex (Korenchevsky and Dennison, 1934b, 1936; Korenchevsky, Dennison, and Levy Simpson, 1935; Korenchevsky, Dennison, and Eldridge, 1937b; Korenchevsky, Hall, and Ross, 1939; Korenchevsky, Hall, and Burbank, 1939). In some cases, however, a considerable enlargement of the kidneys was found after injections of oestradiol esters (Korenchevsky, Hall, and Burbank, 1939).

As injections of oestrogens, however, usually result in arrested growth and therefore decreased body weight, a negative effect when present occurred only in the actual weights of the kidneys; the weights per unit of body weight were usually definitely, often considerably, increased (for details see Korenchevsky, Hall, and Ross, 1939, p. 219). It appears that these changes in the weights of the kidneys might suggest that the effect of oestrogens is of a similar character to that of the male hormones. The

* Dr. Korenchevsky desires to express to the University of Oxford, to Professor E. S. Goodrich, and to Professor J. A. Gunn his own and the Lister Institute's gratitude for the kind hospitality extended to him and his co-workers in the Department of Zoology and Comparative Anatomy and the Nuffield Institute for Medical Research.

fact that the weights per unit of body weight decrease after ovariectomy appears to emphasize this similarity. Histological investigation, however (see later), seems to contradict this suggestion.

SIMULTANEOUS INJECTIONS OF MALE AND FEMALE HORMONES

Since both male and female hormones are normally present in the organism of either sex, it is important to know what are the effects of simultaneous injections of these hormones. It has been shown in the papers mentioned above that when injected simultaneously with oestrogens the male hormones (androsterone, androstanediol, testosterone, and especially its esters, propionate and dipropionate) still exercise their hypertrophying effect on the kidneys. The weights of these organs are, in fact, increased to or towards the level produced by male hormones alone, and in some cases even above it. The same fact was previously found for purified extracts of male hormones from urine simultaneously injected with oestrone (Korenchevsky and Dennison, 1936).

Present Investigation

The total number of normal rats investigated was 148, of which seventy males and twelve females were kept as controls, and thirty-one males and thirty-five females were injected with sex hormones. The total number of gonadectomized rats was 418, of which fifty-nine males and twenty-one females were controls; 142 males and thirty-nine females were injected with male hormones (androsterone, androstanediol, transdehydroandrosterone, Δ^4 -androstenedione, testosterone and its propionate and dipropionate esters); twenty males and thirty-four females were injected with oestrone, oestradiol, or esters of the latter (dipropionate and 3-benzoate-17-*n*-butyrate); and forty-eight males and fifty-five females were injected simultaneously with both male and female hormones.

Technique

The experiments were carried out on 178 rats for twenty-one days and on the remaining 388 rats for two to three and three-quarter months. The majority of the rats were from experiments described in the papers mentioned above, the others are from similar unpublished experiments. Details of the technique have been given in previous papers. The usual microscopical investigation was made of stained sections, the stains used being haematoxylin and eosin, and, when necessary, Masson's trichromic stain and sharlach-red.

In addition, the proximal convoluted tubules were measured, as described below, in ninety-two males (of which seventy-one are included in Table I) and 125 females (Table II), and these rats were injected for two to three and three-quarter months. The groups of rats injected with transdehydroandrosterone and pure testosterone, alone or with oestradiol, were not included in Table I as they showed no definite change in the size of the tubules. All the groups, however, were included in Table II in order to show the accuracy of the method in cases in which no effect was found on tubules. The hormones were dissolved in sesame oil and were injected subcutaneously. Only transverse sections of the proximal convoluted tubules were outlined with the help of a camera lucida on a special paper (magnification $\times 360$). The tracings were then cut out, and the paper replicas thus obtained were weighed before and after cutting away the lumen of the tubules. In the tables only the last figures are given—that is, with the cross-section of the lumen cut

out. Sixty sections of tubules were drawn and weighed from every kidney, and the average of these figures represented the measurement of the tubules for that kidney.

Effect of Gonadectomy

In agreement with the absence of any definite change in weight of kidneys no significant change in the size of the tubules, either by weight or histologically, was found in ovariectomized rats (Table II, compare Groups 5 and 1). In castrated rats the measurements showed a definite decrease in the size of the convoluted tubules (Table I, compare Groups 3 and 1). Thus the definite decrease in the size of the kidneys after castration can be explained by a slight atrophy of the tubular secretory apparatus in these organs.

TABLE I.—*Effect of Sex Hormones on Actual Weights of Kidneys and their Proximal Convoluted Tubules of Male Rats, Normal and Castrated*

Group	Weekly Dose (mg.) of Hormones Injected	No. of Rats in Group	Average Weight of		Average Body Weight of Rats (grammes)
			Kidneys (grammes)	Paper Replicas of Tubules (mg.)	
<i>Normal Rats</i>					
1	Normal controls	16	2.49	8.1	445
2	Testosterone P. 0.75-7.5 ..	6	2.77	8.0	404
<i>Castrated Rats</i>					
3	Castrated controls. . . .	13	1.90	6.5	387
4	Androsterone 7.5 or testosterone P. or D. 0.75-7.5	19	2.51	8.1	367
5	Oestradiol D. or BB. 0.018-0.09	7	1.88	6.3	243
6	Androsterone 7.5 or testosterone P. or D. 0.75-7.5 + oestradiol D. or BB. 0.018-0.09	10	2.59	8.3	301

P.=Propionate. D.=Dipropionate. BB.=3-benzoate-17-*n*-butyrate.

TABLE II.—*Effect of Sex Hormones on Actual Weights of Kidneys and their Proximal Convoluted Tubules of Female Rats, Normal and Ovariectomized*

Group	Weekly Dose (mg.) of Hormones Injected	No. of Rats in Group	Average Weight of		Average Body Weight of Rats (grammes)
			Kidneys (grammes)	Paper Replicas of Tubules (mg.)	
Normal Rats					
1	Normal controls	10	1.65	6.7	250
2	Testosterone P. 0.75-7.5 ..	3	2.41	9.0	260
3	Oestradiol BB. 0.09	4	1.79	6.3	225
4	Testosterone P. 7.5 + oest- radiol BB. 0.09	10	2.34	7.7	216
Ovariectomized Rats					
5	Ovariectomized controls ..	18	1.55	6.4	300
6	Oestradiol D. 0.09-0.2 ..	13	1.54	6.5	197
7	Oestradiol BB. 0.09	12	1.93	6.5	285
8	Androsterone 7.5	3	2.20	7.9	352
9	Androsterone 7.5 + oest- radiol D. or BB. 0.018-0.9	12	2.22	7.1	272
10	Dehydroandrosterone 7.5 ..	4	1.64	6.5	308
11	Dehydroandrosterone 7.5 + oestradiol D. 0.09	4	1.70	7.0	225
12	Testosterone P. 0.75	7	2.08	7.1	314
13	Testosterone P. 0.75 + pro- gesterone 4.5 + oestradiol D. 0.2	11	1.86	7.2	211
14	Testosterone P. 7.5	8	2.49	7.6	277
15	Testosterone P. 7.5 + oest- radiol BB. 0.09	6	2.72	8.6	284

P.=Propionate. D.=Dipropionate. BB.=3-benzoate-17-*n*-butyrate.

Effect of Male Hormones

With the doses used the effect was similar in character, but not in degree, in both sexes. It was clearly nephrotrophic—that is, stimulating the development of the kidneys. In most cases the hormones restored the weight of the kidneys and size of the tubules to normal in males (Table I, compare Group 4 with Groups 1 and 3), but in females the same doses produced supernormal hypertrophy (Table II, compare Groups 2, 8, 12, and 14 with the control groups 1 and 5). Correspondingly, in normal males the effect, if any, on the kidneys was very small (Table I, Group 2), while in normal females it was very pronounced (Table II, Group 2). The increase in the size of the tubules was due chiefly to hypertrophy of the cells, very seldom to their hyperplasia. The lumen of the tubules also was often increased, as the weighing of the paper replicas has shown.

No other definite or constant changes, in particular pathological ones, were found in the kidneys of either sex, except in normal (not gonadectomized) females injected for about four months with testosterone propionate. In these rats slight changes, similar in character to those obtained after oestradiol injections (see below), were observed. No hypertrophic changes were found after injections of transdehydroandrosterone and pure unesterified testosterone, although in some experiments with the latter hormone the kidneys were larger than in the controls.

Effect of Oestrogens

The kidneys of rats injected with pure oestrone or oestradiol for the comparatively short period of twenty-one days were investigated microscopically, but the tubules were not measured. The slight degenerative changes found occurred in a few minute areas of the tubule walls in some rats only.

When oestradiol esters were injected for prolonged periods (Table I, Group 5; Table II, Groups 3, 6, and 7) there were usually no significant or constant changes in the weight of the kidneys. In a few experiments a slight increase was apparent. No change in the size of the tubules was noticed, but pronounced degeneration appeared in the tubules in the boundary between the cortex and the medulla. It is a striking fact that, whereas very pronounced changes occurred in this zone, no changes of similar character or extent were found in the cortex or lower part of the medulla. The changes consisted in extreme dilatation and distortion of the tubules, with atrophy of the cells lining the tubules and usually hyaline degeneration, often followed by the disruption of the extremely atrophic tubular walls. The tubules, through the ruptures, become connected with the peritubular spaces in the connective tissue. These spaces were also distended, seemingly by the same secretion; they were often numerous and irregular in shape, appearing as coalescent cysts. In some cases, due to a complete atrophy of the cells lining the affected tubules, it was difficult to decide where the primary distension started—in the tubules or in the peritubular spaces. The structures were very similar to the cysts described by Letulle (1931) in human kidneys affected by Bright's disease.

Oliver (1939) in his excellent monograph insists that without the microdissection of macerated kidneys it is difficult to diagnose with certainty the formation of numerous small cysts, for which cross-sections of very dilated but continuous tubules might be mistaken. These cysts often occur in Bright's disease, and are formed by

the disruption of the tubules and distension of severed portions.

In the kidneys of our "oestradiol" rats there occurred structures which, with Oliver's warning in mind, we can only with reservations describe as numerous minute cysts. Presumably in the living organ these cysts or dilated tubules were filled with urine, but in our sections they were empty, or contained granular debris of cells or of condensed albumin, or more often hyaline with clearly marked lamination of its substance. Solid hyaline without lamination was found in only a few of the most dilated tubules. Pronounced hyperaemia and in some cases peritubular oedema were other features of the affected areas in these kidneys.

In some kidneys the greater part of this boundary area was degenerated in this way, the cortical tubules appearing to be normal, the only definite change consisting in an increase in the size of the Malpighian glomeruli, indicating a hyperaemic condition of these structures corresponding to the hyperaemia in the affected boundary layer of the kidney. With the largest dose used—0.2 mg. a week—these enlarged glomeruli were not noticed. Since all kinds of tubules are present in the boundary area of the kidneys, the identification of the affected tubules is difficult because of their extreme atrophy and degeneration. Topographic distribution of the tubules in this area, however, suggests that probably Henle's loops were affected and the convoluted tubules were partly affected.

Oliver (1939) has made a similar observation in the kidneys in Bright's disease, in which the affected area was the same boundary layer of the medulla, with cystic changes in the "convoluted and straight tubules."

All these structural changes in the kidneys after prolonged oestrogen administration, together with the absence of any increase in the size of the non-degenerated tubules even when the kidneys themselves were enlarged (Table II, Group 7), indicate that oestrogens have a toxic, not nephrotrophic, effect on these organs.

The enlargement of some of the "oestradiol" kidneys was probably due chiefly to hyperaemia, in some cases accompanied by oedema of the affected areas. True hypertrophy of the kidney, such as was produced by male hormones, was never found after the administration of oestrogens in our experiments. Perhaps the selective changes in Henle's loops are connected with the condensation of urine (and probably of oestrogens in the urine) occurring in these tubules.

Effect of Simultaneous Injections of Male Hormones and Oestrogens, or with Progesterone

When male and female sex hormones were injected simultaneously the hypertrophying effect of the male hormones on the kidneys remained about the same, as judged by weight of kidneys and size of tubules (Table I, Groups 4 and 6; Table II, Groups 12 and 13). However, in females (Table II) the fact that the tubules were smaller in Groups 4 and 9 than in Groups 2 and 8, respectively, may indicate that the increase in weight of the kidneys after these simultaneous injections is partly due to hyperaemia produced by the oestradiol, and is not entirely due to hypertrophy of the organ caused by male hormones.

This is corroborated by histological investigation. The pathological effects of oestradiol (dilatation of the tubules and of the peritubular spaces, the formation of small cysts, and hyperaemia) were prevented or decreased by the neutralizing effect of androsterone and testosterone esters in some rats only; in other rats they were as well

developed as in animals injected with oestradiol esters alone. This was the case even in groups in which the tubules were very large for ovariectomized rats. However, it must be noted that in Group 15 the combined injections were followed by increased hypertrophy of the tubules (Table II) and of their cells. No special protective effect was noticed in Group 13, in which progesterone was injected in addition to the esters of testosterone and oestradiol.

Statistical Analysis of Results Obtained

We are much indebted to E. C. R. Reeve of the Department of Zoology and Comparative Anatomy, University of Oxford, for the following statistical analysis of the results. He writes:

"The data given in Tables I and II were analysed by Fisher's test for comparing the means of small samples (Fisher, 1932, sect. 24, 1). In spite of the small numbers in some groups, the differences stated below to be significant would have occurred in random sampling in less than one case out of a thousand, with one exception (the difference in tubules size between Group 12 and ovariectomized controls of Table II, where the probability is a little greater than one in a thousand). In Table I castrated controls are significantly less than normal controls, and Groups 4 and 6 are significantly greater than castrated controls, in both kidney weight and tubules size. In Table II significant changes in kidney weight and tubules size were found after injection of androsterone and esters of testosterone alone or together with oestradiol esters. Injection of oestradiol benzoate butyrate produced a significant change in kidney weight alone (Table II, Group 7)."

Possible Clinical Application of the Nephrotrophic Properties of Male Hormones

The development and function of every organ in the body are influenced by different chemical factors operating in the organism. From this point of view the results obtained in these experiments strongly suggest that both male and female hormones must be considered as important, perhaps very important, agents in the physiology and pathology of the kidneys. In diseases of these organs a vicious circle easily develops, since pathological kidneys fail to eliminate poisonous metabolic and other products. The accumulation of toxic substances in turn increases the pathological changes in the kidneys. It is obvious that a physiological stimulus might, at least partially, break this vicious circle, and therefore become of considerable therapeutic value.

For therapeutic application androsterone rather than testosterone esters is advised, on the following grounds: (1) androsterone appears to possess nephrotrophic properties of about the same degree as testosterone esters, but (2) its sex effect is definitely weaker than that of testosterone compounds. As it is dangerous and in any case most undesirable to stimulate the sex function in patients suffering from such severe diseases as those of the kidneys, androsterone appears to have an obvious advantage over testosterone for male patients. (3) Androsterone might be tested even on female patients suffering from suitable kidney diseases, since it is physiologically present in the female organism (Callow and Callow, 1938), and at the same time its effect on the female sex organs is comparatively weak (Korenchevsky, 1939).

Unlike the male hormones oestrogens in certain doses have a toxic effect on the kidneys, and therefore their application to patients with kidney diseases is undesirable, and if given in large doses or for prolonged periods might even become dangerous.

Summary

Gonadectomy in males, but not in females, produces small "castration" kidneys.

In both normal and gonadectomized female rats and castrated males the male hormones (androsterone and esters of testosterone) produced true hypertrophy of the kidneys and could therefore be defined as nephrotrophic hormones.

Except for slight pathological changes produced in the kidneys of normal females by large doses, the action of testosterone esters on these organs appears to have no harmful but a definitely beneficial effect.

On the other hand, oestrogens in certain doses easily produced peculiar cyst-like degenerative changes in the kidneys, mostly confined to the boundary layer of the cortex and medulla.

So far as it is permissible from experimental results on rats to advise the therapeutic application of sex hormones to human patients, it is suggested that androsterone, possessing weak sex and strong nephrotrophic properties, should be clinically tested in suitable kidney diseases in males where physiological stimulation of these organs is required.

As androsterone has only a weak action on the female sex organs and occurs normally in the female organism, this hormone might also be cautiously tested in suitable kidney diseases of women.

In women suffering from these diseases, however, the application of oestrogens, especially in large doses or for prolonged periods, would probably increase the pathological changes in the kidneys.

Grants from the Medical Research Council, the Lister Institute, and the Ella Sachs Plotz Foundation have enabled us to carry out this work, and to them our thanks are due. We wish to express our gratitude to Messrs. Organon, Ltd., for the supply of progesterone, and to Messrs. Ciba, Ltd., in particular to Dr. K. Miescher, for all the other hormones.

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At the annual luncheon of the Incorporated Society of Chiropractors on April 13, the president, Mr. J. H. Hanby, said that it was with concern that they had heard of men being taken from the ranks in the Army and trained as so-called chiropractors in the short period of six weeks. The Royal Colleges and the British Medical Association considered that two years was the minimum time required to attain the necessary educational and ethical standard of a medical auxiliary profession. The admission of people with wholly insufficient training meant the deterioration of the standards for which they had fought so hard during the last twenty-five years. Mr. Hugh Lett said that from being not much more than an ill-regulated business twenty-seven years ago the society was now, thanks to its own efforts, a profession, with schools and regulated curriculum. He hoped that generous support would come from medical men and the public for a profession which deserved so much credit.