surgical intervention. The key to the diagnosis is the recognition of sublingual swelling, although the cellulitis may have arisen in either the oral floor or in the submaxillary region.

In this series, cases 1, 2, 3 and 4 are typical Ludwig's angina. Case 5 is representative of one of the clinical pictures over which the Nelaton and Delorme factions waged so much controversy. In the earlier stages, case 5 would never have been confused with Ludwig's angina, but on the twenty-sixth day of the disease, all criteria of the diagnosis were fully satisfied.

SUMMARY

In the early stages of Ludwig's angina, penicillin will control the infection in a high percentage of cases and greatly reduce the number requiring early surgery.[8] This series of five cases is not sufficiently large to permit the generalization that penicillin should replace surgical treatment.

It is evident that no therapeutic measure will ever replace tracheotomy for the patient with serious mechanical respiratory embarrassment. It must be clearly understood that adequate surgical decompression is demanded at the first indication of failure of the agent to control the infection.

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RÉSUMÉ

Dans cinq cas d'angine de Ludwig le traitement précoce par la pénicilline a évité l'intervention chirurgicale, qui est la thérapeutique habituelle de cette maladie. Il est manifeste que la trachéotomie ne peut être évitée lorsqu'il existe un obstacle mécanique à la respiration, mais il est probable que la pénicilline, administrée selon la technique mentionnée ici réduira considérablement le nombre des interventions chirurgicales dans l'angine de Ludwig. JEAN SAUCIER

## STUDIES CONCERNING THE EFFECTS OF VARIOUS HORMONES UPON RENAL STRUCTURE

By Hans Selye, M.D., Ph.D., D.Sc., F.R.S.C., Helen Stone, Kai Nielsen and Charles P. Leblond, M.D., Ph.D.

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IT is becoming increasingly more obvious that hormonal stimuli exert important effects upon the structure of the kidney. It has been known for some time that thyroxin causes renal enlargement in a variety of animal species[1-7] and more recently it has been demonstrated that various steroid hormones and anterior pituitary preparations likewise exert an important influence upon renal structure.

The effects of these hormone preparations may be roughly subdivided into two distinct types; the renotropic action and the nephrosclerotic action. The renotropic action is a purely stimulating one, mainly characterized by hyperplasia and hypertrophy of the tubular cells. The nephrosclerotic action on the other hand is definitely damaging to the kidney; it manifests itself by a more or less malignant type of nephrosclerosis, with hyalinization of the renal glomeruli and arterioles as well as the formation of hyaline casts which obstruct the tubular lumina. Experimental nephrosclerosis produced by hormones is usually accompanied by periarteritis nodosa in extrarenal blood vessels. Among the hormone preparations so far examined testosterone and many of its derivatives proved to possess a pure renotropic action[8-10] while desoxycorticosterone acetate (D.C.A.) exhibits only nephrosclerotic properties.[11-14] Extracts prepared from the anterior lobe of cattle pituitaries are apparently endowed with both nephrosclerotic and renotropic potency and up to the present time it has not been possible to separate the crude preparations into purified fractions exhibiting the nephrosclerotic or the renotropic effect exclusively.[15-17]

The nephrosclerotic action of D.C.A. and of anterior lobe preparations is most obvious in unilaterally nephrectomized animals kept on a high sodium chloride intake. On the other hand neither unilateral nephrectomy nor a high sodium chloride intake appear to cause any significant increase in the kidney-stimulating potency of the renotropic steroids and anterior

lobe preparations. The selective sensitization of experimental animals to nephrosclerosis, by unilateral nephrectomy and high NaCl intake therefore proved to be a useful procedure for the detection of the nephrosclerotic property.

The experiments to be reported in this communication were performed to examine the effect upon the rat kidney of thyroxin, anterior lobe preparations, D.C.A., and methyl-testosterone, both singly and in various combinations. In most instances these hormones and hormone combinations were studied comparatively in intact animals kept on a normal NaCl intake and in unilaterally nephrectomized rats given abnormally high amounts of NaCl. It was hoped that systematic investigations of this type would reveal the optimum experimental conditions for the selective production of nephrosclerosis on the one hand, and of the renotropic effect on the other. The production of experimental nephrosclerosis is of special interest since it strikingly resembles the corresponding spontaneous disease of man, and elucidation of the pathogenetic mechanism responsible for the production of the former promised to throw some light upon the etiology and pathogenesis of the latter. Conversely, the renotropic effect interested us because we hoped stimulation of normal kidney growth might furnish us with important leads concerning the therapy of clinical conditions due to a destruction of renal tissue by disease.

## EXPERIMENTAL

In the first series eight groups each consisting of 10 hooded castrate male rats ranging in weight from 90 to 110 gm. were unilaterally (left side) nephrectomized. In order to control the intake of NaCl, all groups were given 2 c.c. of 10% NaCl by gavage twice daily, and tap water *ad lib.* Group I acted as controls and was otherwise untreated; Group II received 200 $\gamma$ of thyroxin as a finely ground suspension in 0.1 c.c. of distilled water; Group III received 0.4 c.c. of an anterior pituitary brei made by mixing 50 gm. of finely ground beef anterior pituitary tissue with 250 c.c. of physiological saline; in Group IV one pellet of D.C.A. compressed at a pressure of 1,000 lbs. per cm.$^2$ and weighing about 45 mgm. was subcutaneously implanted into each animal; Group V received both thyroxin and anterior lobe brei in the same manner and dosage as Groups II and III; Group VI received both a D.C.A. pellet and the anterior lobe brei in the same manner and dosage as Groups III and IV; Group VII received both a D.C.A. pellet and thyroxin in the same manner and dosage as Groups II and IV and Group VIII received all three of these treatments (D.C.A., thyroxin and anterior lobe brei) simultaneously.

In this, as in the other series to be reported in this paper, all animals were fed exclusively on "Purina fox chow" and all surgical operations (including the implantation of pellets) were performed within the 24 hrs. preceding the initiation of injections. After 11 days of treatment the animals were killed, and their organs fixed in Suza mixture after which they were weighed and histologically examined.

In Table I as in all subsequent tables we listed the average initial and final body weight (the latter with its standard error) of the animals and the average absolute weight of the kidney and heart (with standard error). Since the weight of the kidney and heart is largely dependent upon the body weight, these two organ weights are also expressed as a percentage of

TABLE I.

ORGAN CHANGES IN CASTRATE MALE UNILATERALLY NEPHRECTOMIZED HOODED RATS MAINTAINED ON A HIGH NaCl INTAKE AND TREATED WITH VARIOUS HORMONES FOR A PERIOD OF 11 DAYS

| *Group* | *Treatment* | *Body weight (initial) (gm.)* | *Body weight (final) (gm.)* | *Kidney weight ± standard error (gm.)* | *Kidney weight as % of body weight* | *% of incidence* | | *Heart weight ± standard error (gm.)* | *Heart weight as % of body weight* | *% of cardiac lesions* | *Adrenal weight (gm.)* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Nephrosclerosis* | *Kidney pelvis œdema* | | | | |
| I. | Controls | 99 | 121±4 | 0.838±36 | 0.69 | 0 | 0 | 0.455±16 | 0.37 | 0 | 0.027 |
| | | | | | *% deviation from control* | | | | *% deviation from control* | | *% deviation from control* |
| II. | Thyroxin | 94 | 110±6 | 1.183±54 | 55 | 9 | 0 | 0.607±22 | 49 | 19 | 18 |
| III. | Hyp. prep. | 94 | 131±5 | 0.968±54 | 6 | 14 | 0 | 0.532±23 | 8 | 0 | 48 |
| IV. | D.C.A. | 94 | 108±6 | 1.064±18 | 42 | 13 | 80 | 0.504±22 | 24 | 13 | 37 |
| V. | Thyroxin + Hyp. prep. | 99 | 137±5 | 1.513±75 | 59 | 4 | 50 | 0.702±22 | 38 | 8 | 115 |
| VI. | D.C.A. + Hyp. prep. | 94 | 128±5 | 1.384±58 | 56 | 25 | 80 | 0.530±21 | 11 | 8 | 104 |
| VII. | Thyroxin + D.C.A. | 93 | 106±6 | 1.251±87 | 71 | 22 | 50 | 0.563±60 | 43 | 17 | 30 |
| VIII. | Thyroxin + D.C.A. + Hyp. prep. | 94 | 113±5 | 1.445±98 | 85 | 39 | 100 | 0.587±27 | 40 | 28 | 122 |

the total body weight. In the case of the normal control animals, the percentual kidney and heart weight respectively are expressed as such. In all experimental groups, however, the percentual deviation of the percentual weight from that of the controls is given. It was felt that it is more instructive to list the deviation rather than the absolute percentual weight since it is the change in weight (the deviation from the normal) that is of importance for the evaluation of our findings and not the weight itself. The absolute percentual kidney weight may, however, readily be calculated from the data of the table.

The histological changes in the kidney and heart are difficult to tabulate in a quantitative manner, but in order to furnish comparable average data the changes were expressed in a scale of + to +++ indicating increasingly pronounced degrees of inflammatory and degenerative lesions. In the columns headed "% incidence of nephrosclerosis" and "% of cardiac lesions" respectively, the intensity of these changes is expressed as a percentage of the theoretical maximum, which would be +++ in all cases. Thus, +++ lesions in all animals of the group are expressed as 100% while 33% indicates + in all animals or +++ in one-third of the animals, etc. A more detailed histological description of the changes is given below in the text. The absolute average adrenal weight is listed in the case of the controls while in all experimental groups the percentual deviation from this normal control weight is given.

In many of our animals marked œdema or actual inflammatory changes have been seen in the connective tissues surrounding the renal pelvis. These are difficult to estimate even in an approximate manner using a + to +++ scale. Without making serial sections through the entire pelvic region it would be extremely difficult to judge the extent of inflammation and œdema. Such serial sections have not been prepared, hence in our table changes in the renal pelvis (œdema or inflammation) are indicated by numbers referring to the percentual incidence irrespective of severity.

Perusal of Table I clearly indicates that all types of treatment (Groups II to VIII) caused an increase in kidney weight as compared to the controls (Group I). The absolute kidney weight was largest in Group V under the simultaneous influence of thyroxin and the hypophyseal preparation. But when the kidney weight is expressed as a percentage of the total body weight, simultaneous treatment with thyroxin, D.C.A., and the hypophyseal preparation (Group VIII) proved to cause the greatest increase in the renal weight, due to the fact that the body weight did not rise as markedly as that of the animals of Group V. It will also be noted that, in this eleven-day experiment, the renotropic action of thyroxin and of the hypophyseal preparation was merely additive in the case of combined administration of the two preparations (Group V). The nephrosclerotic effect of D.C.A. (Group IV) was somewhat increased by simultaneous treatment with the pituitary preparation (Group VI) or thyroxin (Group VII) and was most pronounced in the group receiving both thyroxin and the hypophyseal preparation in addition to D.C.A. (Group VIII).

Œdema or actual inflammatory lesions in the fat tissue surrounding the renal pelvis were seen in a significant percentage of the animals of all groups except the first three.

The heart weights approximately paralleled the kidney weights in the various groups, while the myocardial and endocardial lesions roughly paralleled the degree of nephrosclerosis.

It is rather interesting to note—though difficult to explain—that the adrenal weights were highest in Groups V, VI, and VIII, that is to say, in those animals which received the hypophyseal preparation in combination with thyroxin, D.C.A. or both of these compounds. This is all the more surprising since in intact (not partially nephrectomized) animals kept on a normal NaCl intake, D.C.A. causes compensatory atrophy of the adrenals. Even in this experiment D.C.A. caused no very significant adrenal enlargement when given by itself (Group IV) and the adrenotropic activity of the hypophyseal preparation in itself was likewise rather moderate, causing only a 48% rise above the control adrenal weight (Group III). The adrenotropic action of the anterior lobe preparation when given in combination with thyroxin (Group V) was greater than could be expected on the basis of a mere summation of the adrenal stimulating effect of these two hormone preparations given singly (Groups III and V). This potentiation of the adrenotropic effect of the anterior lobe preparation was also manifest in all other experimental series to be reported in this paper (see Tables II, III and IV).

TABLE II.

ORGAN CHANGES IN FEMALE UNILATERALLY NEPHRECTOMIZED ALBINO RATS, MAINTAINED ON A HIGH NaCl INTAKE AND TREATED WITH VARIOUS HORMONES FOR A PERIOD OF 20 DAYS

| Group | Treatment | Body weight (initial) (gm.) | Body weight (final) (gm.) | Kidney weight ± standard error (gm.) | Kidney weight as % of body weight | % of incidence | | Heart weight ± standard error (gm.) | Heart weight as % of body weight | % of cardiac lesions | Adrenal weight (gm.) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Nephrosclerosis | Kidney pelvis œdema | | | | |
| I. | Controls | 121 | 159±6 | 1.030±37 | 0.65 | 0 | 0 | 0.568±25 | 0.35 | 0 | 0.044 |
| | | | | | % deviation from control | | | | % deviation from control | | % deviation from control |
| II. | Thyroxin | 121 | 164±6 | 1.310±49 | 23 | 0 | 0 | 0.756±29 | 31 | 3 | 36 |
| III. | Hyp. prep. | 121 | 224±7 | 1.540±43 | 6 | 0 | 90 | 0.854±23 | 9 | 17 | 89 |
| IV. | D.C.A. | 121 | 147±9 | 1.176±75 | 23 | 28 | 100 | 0.723±37 | 38 | 28 | 7 |
| V. | Thyroxin + Hyp. prep. | 120 | 193±11 | 2.485±178 | 97 | 42 | 100 | 1.095±58 | 60 | 33 | 184 |
| VI. | D.C.A. + Hyp. prep. | 121 | 202±13 | 1.983±148 | 51 | 46 | 100 | 0.941±53 | 31 | 52 | 93 |
| VII. | Thyroxin + D.C.A. | 121 | 153±8 | 1.739±102 | 74 | 61 | 100 | 0.863±50 | 60 | 66 | 46 |
| VIII. | Thyroxin + D.C.A. + Hyp. prep. | 120 | 192±8 | 2.628±150 | 110 | 68 | 100 | 1.098±52 | 63 | 69 | 207 |
| IX. | Progesterone | 112 | 153±6 | 1.110±41 | 11 | 0 | 0 | 0.563±22 | 3 | 0 | −2 |
| X. | Thyroxin + Progesterone | 111 | 151±5 | 1.438±39 | 46 | 0 | 0 | 0.698±8 | 31 | 0 | 20 |

In the second series 110 female albino rats ranging in weight from 100 to 140 gm., were divided into 10 groups. Each group consisted of 10 animals but in Group VIII 20 animals were used because of the expected high mortality. All animals were unilaterally nephrectomized and, as in the first series, the NaCl intake was controlled by giving 3 c.c. of 10% NaCl twice daily by gavage; tap water was allowed *ad lib.*

Groups I to VIII received the same treatment, and in the same dosage, as the correspondingly numbered groups in the first series; Group IX received 5 mgm. of progesterone suspended in 0.1 c.c. of distilled water subcutaneously twice daily; Group X was given the same dose of progesterone as Group IX in combination with 200 γ of thyroxin. The animals were killed after 20 days of treatment; their organs were fixed in Suza, weighed and histologically examined.

It will be noted that the first eight groups of this second experimental series represent essentially a repetition of the first series, the only difference being that female albino rather than castrate male hooded rats, were used in order to establish whether female animals of a somewhat different strain would react essentially in the same manner. It will be recalled that male animals are unsuited for this type of experimentation because they are more resistant to nephrosclerosis; furthermore, having an important source of endogenous renotropic steroids in their testes, they do not lend themselves to the bioassay of this latter activity, therefore in all our relevant experiments either male castrate or female rats were used.

The last two groups (IX and X) were added in order to establish whether progesterone has any important renotropic or nephrosclerotic activity either when given alone or in combination with thyroxin.

Perusal of Table II indicates that in this somewhat more prolonged experiment, which lasted 3 weeks, the response to the different types of treatment was essentially the same—though more pronounced—as in the corresponding groups of the first series. In the present series combined treatment with thyroxin and the hypophyseal preparation (Group V) caused the second greatest, while treatment with thyroxin, D.C.A. and hypophyseal preparation (Group VIII) caused the greatest absolute and percentual increase in renal weight. It is remarkable that in the short period of 20 days the kidney weight was more than twice that of the controls in this group. The nephrosclerosis, however, was likewise highest in Group VIII so that the enlargement of kidney size was probably not accompanied by an improvement in renal function. On the other hand, in Group V under the combined influence of thyroxin and the hypophyseal preparation nephrosclerosis, though still very pronounced, was not as marked as in Group VIII. It will also be noted

that while thyroxin caused no nephrosclerosis (Group II), and D.C.A. alone (Group IV) caused only 28% of the possible maximum, the combined administration of thyroxin and D.C.A. (Group VII) resulted in a considerable increase to 61% in the nephrosclerotic activity of the latter compound.

In this 20-day experiment a high incidence of kidney pelvis œdema or inflammation was noted in all groups but the controls and the merely thyroxin treated animals (Groups I and II).

The heart weight tended to parallel the kidney weight in this as in the first experimental series and the cardiac lesions approximately paralleled the nephrosclerosis in the various groups.

As in the first series the largest adrenal weights were noted in the groups treated with the hypophyseal preparation in combination with thyroxin (Group V), D.C.A. (Group VI), or both of these preparations (Group VIII). The synergistic effect of D.C.A. alone (Group VI) was not particularly marked in the present series but thyroxin and thyroxin plus D.C.A. (Groups V and VIII respectively) proved to cause a much more pronounced adrenal enlargement than the hypophyseal preparation alone (Group III). It may be said that histological examination of these adrenals indicates that the enlargement is entirely due to cortical hyperæmia, hyperplasia and hypertrophy both in the first and in the second series. From these observations we may conclude that at least thyroxin and perhaps also D.C.A. exert a considerable synergistic effect upon the corticotropic activity of our hypophyseal preparation.

As regards progesterone given either by itself or in combination with thyroxin, our results clearly indicate that unlike in the chick[14] progesterone causes no nephrosclerosis in the rat, at least under the conditions under which it was tested in these experiments. Indeed, even the simultaneous administration of thyroxin—which so considerably enhances the nephrosclerotic action of D.C.A.—is ineffective in revealing any nephrosclerotic potency in the chemically so closely related progesterone.

Subsequently, two additional series of experiments (Series III and Series IV) were undertaken specifically in order to determine the optimum experimental conditions for the renotropic effect. Earlier experiments, mentioned in the introductory part of this paper, indicated that methyl-testosterone is one of the most active renotropic steroids. We therefore decided to substitute methyl-testosterone for D.C.A. in our last two experiments which were otherwise carried out essentially in the same manner as those of Series I and II.

In the third series, 96 castrate male albino rats, ranging in weight from 90 to 140 gm., were divided into 8 groups of 12 animals each. All rats were unilaterally nephrectomized and given 3 c.c. of 10% NaCl by gavage twice daily; tap water was allowed *ad lib.*

Groups II, III and V received the same treatment as the corresponding groups in series I and II; in Group IV each animal was implanted with one pellet of methyl-testosterone (Me-testo) weighing about 45 mgm.; Group VI received one pellet of Me-testo and 200 $\gamma$ of thyroxin; Group VII received a similar Me-testo pellet and 0.4 c.c. of the anterior lobe preparation twice a day; Group VIII was given all three of these treatments simultaneously. The animals were sacrificed after 20 days of treatment and their organs were fixed in Suza weighed and histologically examined.

Sub-group 1 of each group in Table III represents the data obtained from 6 of the animals of each group in which food was withdrawn 16 hours before killing in order to determine the effect of fasting upon the organs influenced by the hormone treatment. Sub-group 2 summarizes the data obtained in the remaining fed animals. The final body weight in both these sub-groups was determined under equal conditions before food was withdrawn in sub-group 1.

It will be recalled that unilateral nephrectomy and administration of large amounts of NaCl considerably enhance the nephrosclerotic activity of hormone preparations, but do not exert any very pronounced sensitizing effect upon their renotropic action. It is for this reason that the final experiment was performed on intact rats receiving only tap water to drink. It was felt that under these conditions the renotropic or kidney-stimulating effect of the hormones and hormone combinations would probably manifest itself under optimum conditions so that increased production of healthy kidney tissue would result.

For this purpose in a fourth series, 96 castrate male albino rats ranging in weight from 90 to 140 gm., were divided into 8 groups of 12 animals and given tap water *ad lib.* All groups received the same hormone treatment as the correspondingly numbered groups in Series III, but unlike the latter they received no NaCl treatment and were not partially nephrec-

TABLE III.

ORGAN CHANGES IN CASTRATE MALE UNILATERALLY NEPHRECTOMIZED ALBINO RATS MAINTAINED ON A HIGH NaCl INTAKE AND TREATED WITH VARIOUS HORMONES FOR A PERIOD OF 20 DAYS

| Group | Treatment | | Body weight (initial) (gm.) | Body weight (final) (gm.) | Kidney weight ± standard error (gm.) | Kidney weight as % of body weight | % of incidence: Nephrosclerosis | % of incidence: Kidney pelvis œdema | Heart weight ± standard error (gm.) | Heart weight as % of body weight | % of cardiac lesions | Adrenal weight (gm.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I. | Controls | 1 | 114 | 157±7 | 1.126±62 | 0.71 | 0 | 0 | 0.612±27 | 0.39 | 0 | 0.041 |
| | | 2 | | 165 | 1.258 | 0.76 | | | 0.598 | 0.36 | | 0.039 |
| | | | | | | % deviation from control | | | | % deviation from control | | % deviation from control |
| II. | Thyroxin | 1 | 113 | 175±8 | 1.493±63 | 20 | 3 | 0 | 0.812±23 | 18 | 3 | 12 |
| | | 2 | | 170 | 1.636 | 26 | | | 0.798 | 31 | | 49 |
| III. | Hyp. prep. | 1 | 111 | 212±7 | 1.868±124 | 24 | 12 | 10 | 0.957±42 | 15 | 4 | 122 |
| | | 2 | | 198 | 2.399 | 58 | | | 0.852 | 19 | | 154 |
| IV. | Me-Testo | 1 | 111 | 196±8 | 1.392±64 | 0 | 0 | 0 | 0.681±33 | −10 | 0 | −27 |
| | | 2 | | 160 | 1.474 | 21 | | | 0.639 | 11 | | 8 |
| V. | Thyroxin + Hyp. prep. | 1 | 112 | 209±11 | 2.814±227 | 88 | 12 | 30 | 1.061±69 | 30 | 43 | 175 |
| | | 2 | | 166 | 2.926 | 132 | | | 1.066 | 78 | | 256 |
| VI. | Thyroxin + Me-Testo | 1 | 114 | 159±9 | 1.548±121 | 37 | 0 | 0 | 0.748±50 | 20 | 3 | −7 |
| | | 2 | | 156 | 1.723 | 45 | | | 0.759 | 36 | | 11 |
| VII. | Hyp. prep. + Me-Testo | 1 | 114 | 223±14 | 2.077±153 | 31 | 12 | 30 | 0.925±26 | 5 | 10 | 105 |
| | | 2 | | 229 | 2.927 | 67 | | | 1.029 | 25 | | 143 |
| VIII. | Thyroxin + Me-Testo + Hyp. prep. | 1 | 114 | 201±14 | 2.747±139 | 91 | 67 | 20 | 0.978±38 | 23 | 40 | 263 |
| | | 2 | | 203 | 2.807 | 81 | | | 0.903 | 22 | | 210 |

TABLE IV.

ORGAN CHANGES IN CASTRATE MALE NOT UNILATERALLY NEPHRECTOMIZED ALBINO RATS MAINTAINED ON A DIET CONTAINING A NORMAL AMOUNT OF NaCl AND TREATED WITH VARIOUS HORMONES FOR A PERIOD OF 20 DAYS

| Group | Treatment | | Body weight (initial) (gm.) | Body weight (final) (gm.) | Kidney weight ± standard error (gm.) | Kidney weight as % of body weight | % of incidence: Nephrosclerosis | % of incidence: Kidney pelvis œdema | Heart weight ± standard error (gm.) | Heart weight as % of body weight | % of cardiac lesions | Adrenal weight (gm.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I. | Controls | 1 | 111 | 176±10 | 1.468±85 | 0 83 | 0 | 0 | 0.612±32 | 0.34 | 0 | 0.035 |
| | | 2 | | 174 | 1.670 | 0.90 | | | 0.590 | 0.34 | | 0.038 |
| | | | | | | % deviation from control | | | | % deviation from control | | % deviation from control |
| II. | Thyroxin | 1 | 113 | 153±6 | 1.589±45 | 24 | 0 | 0 | 0.687±11 | 32 | 0 | 20 |
| | | 2 | | 183 | 2.115 | 28 | | | 0.826 | 32 | | 16 |
| III. | Hyp. prep. | 1 | 113 | 227±9 | 2.164±126 | 14 | 0 | 0 | 0.879±64 | 15 | 0 | 126 |
| | | 2 | | 216 | 2.358 | 21 | | | 0.807 | 9 | | 127 |
| IV. | Me-Testo | 1 | 111 | 178±6 | 1.654±26 | 12 | 0 | 0 | 0.658±5 | 9 | 0 | −17 |
| | | 2 | | 166 | 1.836 | 22 | | | 0.585 | 3 | | −3 |
| V. | Thyroxin + Hyp. prep. | 1 | 113 | 214±4 | 2.656±115 | 49 | 0 | 10 | 0.995±45 | 35 | 8 | 280 |
| | | 2 | | 190 | 3.120 | 82 | | | 0.970 | 50 | | 323 |
| VI. | Thyroxin + Me-Testo | 1 | 114 | 175±7 | 1.993±51 | 37 | 0 | 0 | 0.792±46 | 32 | 0 | 14 |
| | | 2 | | 185 | 2.287 | 37 | | | 0.738 | 15 | | −5 |
| VII. | Hyp. prep. + Me-Testo | 1 | 113 | 227±8 | 2.417±71 | 28 | 0 | 0 | 0.932±21 | 21 | 0 | 106 |
| | | 2* | | .. | .. | .. | | | .. | .. | .. | .. |
| VIII. | Thyroxin + Me-Testo + Hyp. prep. | 1 | 111 | 231±6 | 2.884±202 | 50 | 0 | 70 | 0.979±72 | 24 | 0 | 214 |
| | | 2 | | 230 | 3.554 | 71 | | | 1.010 | 29 | | 192 |

*Owing to intercurrent disease all animals died in group 2.

tomized. The experiment ran simultaneously with Series III for twenty days, and the conditions under which the animals were sacrificed were also the same as in Series III. It will be noted that in Table IV which summarizes our pertinent data, the figures in the columns headed "Kidney Weight" refer to the combined weight of two kidneys.

The results of Tables III and IV may best be discussed conjointly. It appears that under the experimental conditions and at the dose level employed in these experiments methyl-testosterone in itself did not exert any significant renotropic effect. When given in conjunction with thyroxin, the renotropic effect of methyl-testosterone was apparently merely added to that of the former compound. On the other hand, the synergism between the renotropic action of thyroxin and the hypophyseal preparation appears to have been more than merely additive as judged by the results of both Series III and Series IV. In this instance, a true potentiation appears to have occurred, inasmuch as the percentual kidney weight increase in Group V of both experiments is greater than could be expected on the basis of mere summation of the increases noted in Groups II and III. It is of special interest to note in this connection, that this very powerful renotropic combination of hormones caused only traces of nephrosclerosis and renal pelvis œdema in a few animals in either of the two series. In agreement with expectations, nephrosclerosis and œdema or inflammation in the renal pelvis region occurred only in the groups in which the hypophyseal preparation was injected; they were more obvious in Series III (sensitization by unilateral nephrectomy and NaCl) than in Series IV. The fact that in Group VIII of Series III the incidence of nephrosclerosis was high, may be attributed to the fact that thyroxin sensitizes both to the nephrosclerotic and to the renotropic effect of the various hormone preparations. Even combined treatment with thyroxin, methyl-testosterone and the hypophyseal preparations (Group VIII) however, caused no nephrosclerosis in any of the intact animals of Series IV which received a normal NaCl intake.

The significance of the kidney pelvis changes is not fully understood as yet, but they appear to develop only in animals treated with compounds capable of eliciting nephrosclerosis, such as D.C.A. or the hypophyseal preparation. This effect is also reminiscent of the nephrosclerotic action in that it is more evident in unilaterally nephrectomized NaCl-treated than in intact animals, yet there is no very close quantitative relationship between the intensity of the renal pelvis lesions and the degree of nephrosclerosis in individual animals.

Comparison of the organ weights in subgroups 1 and 2 of the various groups in Series III and IV indicates that a fasting period of even only 16 hours suffices to reduce the weight of the enlarged kidneys and indeed this reduction tends to be greatest in the animals with the most marked renal enlargement. The heart and adrenal weights show no consistent change attributable to this short fast.

### HISTOLOGICAL OBSERVATIONS

In the experimental part of this publication, we mentioned structural changes in the kidneys, heart and adrenals only from a quantitative point of view, that is, without entering into histological details. It is of some interest, however, to give a brief description of the microscopical changes observed. This may best be done conjointly for the various groups since the organ changes were essentially similar in many of the experiments.

As regards the kidney we distinguish two main types of action, namely, the renotropic and the nephrosclerotic. The renotropic effect, as we understand it, is a purely kidney-growth-stimulating action, such as is manifested in the present experimental series by methyl-testosterone and thyroxin. The hypophyseal preparation exerted a prevailingly renotropic action, but in high dosages and when administered to animals sensitized by unilateral nephrectomy and NaCl, this was sometimes accompanied by nephrosclerosis. Methyl-testosterone stimulates the tubular portion of the nephron exclusively,—as do most if not all the renotropic steroids—without causing any significant increase in glomerular size. On the other hand thyroxin, as well as the hypophyseal preparations, elicites a significant increase in the diameter of the glomeruli as well as a hyperplasia and hypertrophy of the tubules.

The most pronounced renotropic effect was seen in Group V of the four experimental series, that is to say, in the animals treated simultaneously with thyroxin and the hypophyseal

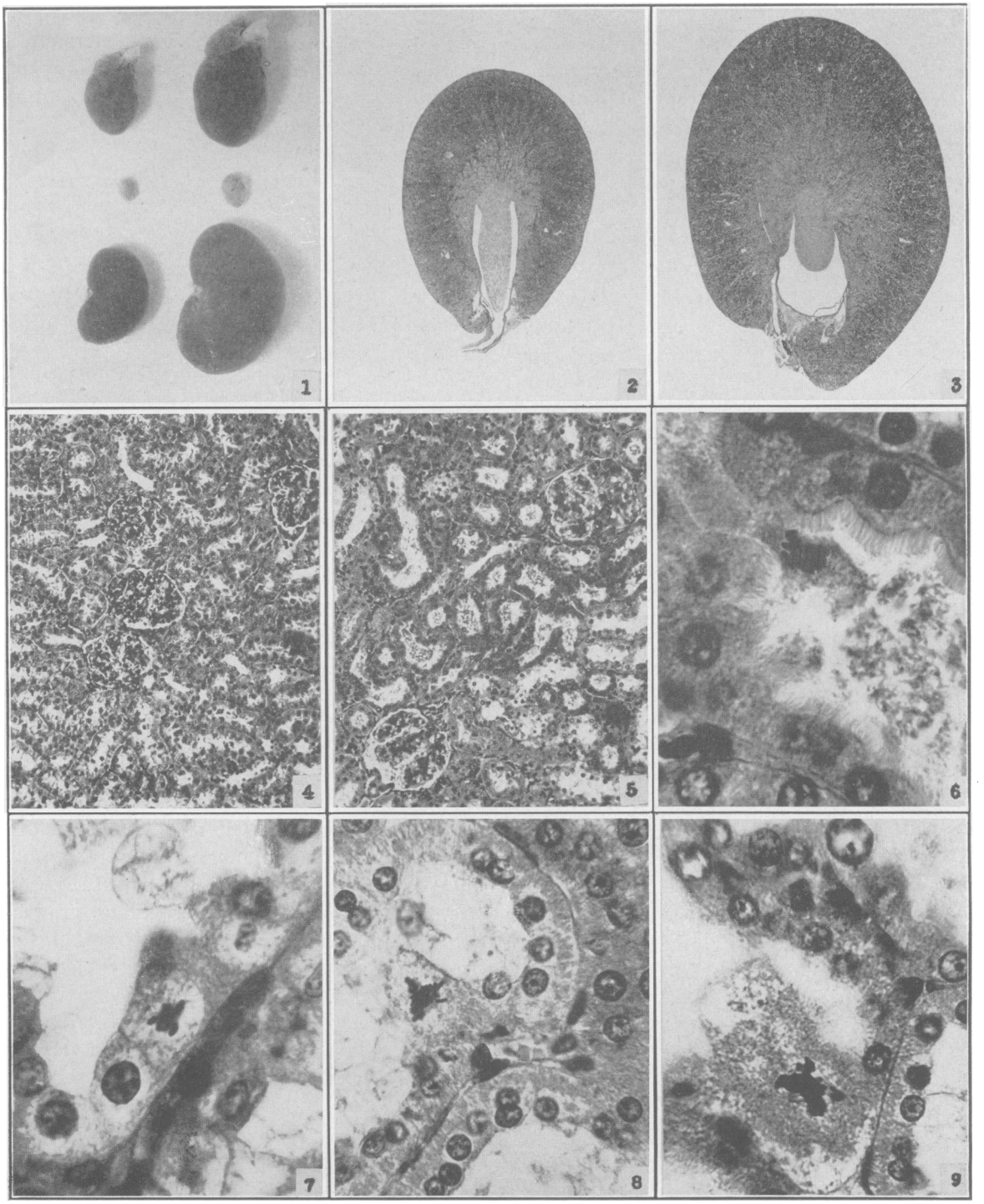
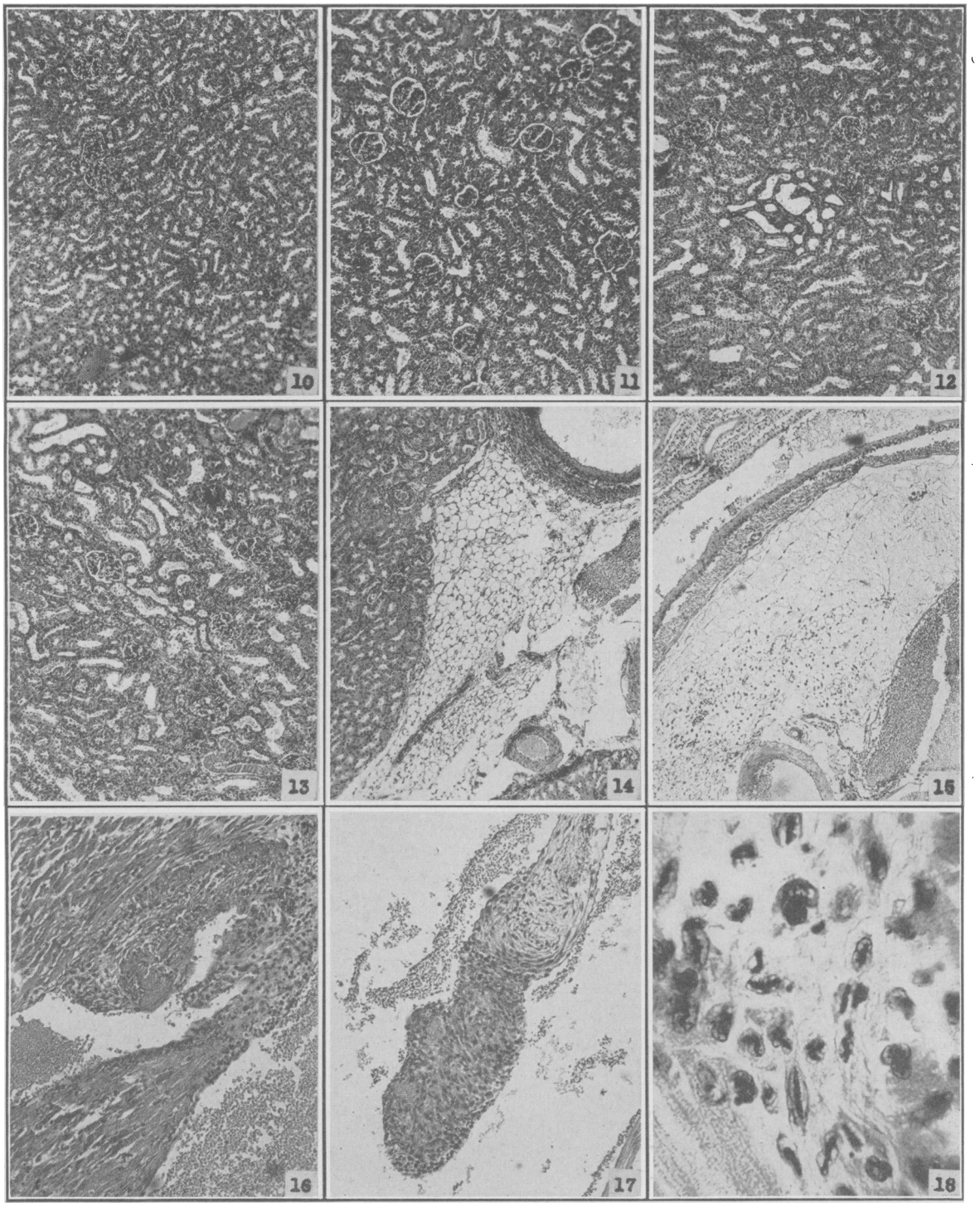



**Fig. 1**—Macroscopic aspect of heart, adrenal and kidney of a control (left) and an experimental (right) animal; the latter being treated with the hypophyseal preparation and thyroxin for a period of 20 days (from experimental Series II). **Figs. 2 and 3**—Low magnification of a cross-section through the kidney of a control (Fig. 2) and an experimental (Fig. 3) animal, the latter being treated with the hypophyseal preparation and thyroxin for a period of 20 days. Fig. 3 shows the enormous enlargement, especially of the cortical portions of the kidney, under the influence of the renotropic hormone combination (from experimental Series III). **Figs. 4 and 5.**—Medium magnification of the renal cortex of a control (Fig. 4) rat and a rat treated with the hypophyseal preparation and thyroxin for a period of 20 days (Fig. 5). In the latter note the hypertrophy and hyperplasia of the epithelial cells lining the tubules, regular and proportionate distension of all tubular lumina and enlargement of the renal glomeruli, but no signs of nephrosclerosis (from experimental Series II). **Figs. 6, 7, 8 and 9.**—Oil immersion view of various fields from the kidney of a rat having received the hypophyseal preparation and thyroxin for a period of 20 days (from experimental Series III). Note comparatively normal mitotic division in one cell and bud-like protrusion of the cytoplasm into the tubular lumen in another cell. This latter cytoplasmic change resembles apocrine secretion and is comparatively common in the kidneys of animals treated with anterior lobe preparations. Fig. 7 shows a cell in the process of mitotic division, about to be discharged into the tubular lumen. Fig. 8 illustrates a gigantic cell in mitosis apparently also ready to be shed off into the lumen. Note also several binucleated cells and great anisocytosis within this field. Compare, for instance, size of nuclei in the convoluted tubule at the lower right of the field with the giant nuclei on the upper border of the photograph in Fig. 9.




**Figs. 10, 11, 12 and 13.**—Various stages in the process of nephrosclerosis (experimental Series II). Fig. 10 shows the size of the convoluted tubules and glomeruli in the normal control kidney. Fig. 11 reveals the slight enlargement of tubules and glomeruli caused by thyroxin alone. Fig. 12 illustrates the very mild nephrosclerosis (dilatation of a few tubules with some intertubular sclerosis) elicited by the short treatment with D.C.A. alone and Fig. 13 the more advanced nephrosclerosis (tubular casts, many dilated tubules, some glomerular sclerosis) elicited by simultaneous treatment with thyroxin and D.C.A. **Figs. 14 and 15**—Normal connective and fat tissue in the renal pelvis of a control animal (Fig. 14) and œdematous connective tissue with round cell infiltration in that of a rat treated with hypophyseal preparation, thyroxin and D.C.A. (Fig. 15) (from experimental Series II). **Figs. 16, 17 and 18.**—Various stages of the endo- and myocarditis seen in rats treated with the anterior pituitary preparation, thyroxin and D.C.A. (from experimental Series II). Fig. 16 shows endocardial vegetation between capillary muscles. There is some proliferating, giant cell containing granulomatous tissue near the base of the vegetation where it is attached to the endocardium while the lumenward directed free surface (lower part of picture) consists entirely of a hyalinized homogeneous mass. Fig. 17 illustrates a granulomatous excrescence on the free edge of the mitral valve. Fig. 18 shows a multitude of the typical "Antischkow myocytes" or "caterpillar cells". Note the caterpillar-like arrangement of the chromatin in the elongated nuclei.

preparations. In these groups as well as in Group VIII of Series III and IV—in which in addition to thyroxin and hypophyseal preparations, methyl-testosterone was given—the tubular epithelia and the glomeruli were not only enlarged but there were also signs of excessive mitotic proliferation in the various parts of the convoluted tubules. These mitoses were frequently atypical and in many cases appear to have ended in the death of the cells. Many of the tubular epithelial cells in which mitoses were seen, were gigantically enlarged and contained chromosomes and spindles of unusual size. Some of the dividing cells were observed to be discharged into the tubular lumina. There was also a considerable degree of anisocytosis and numerous binuclear or even polynuclear epithelial cells were seen among the normal lining cells of the tubules (see Figs. 1 to 9).

All these phenomena are probably an indication of an excessive proliferative activity, which takes place under the influence of overdosage with potent renotropic hormones and hormone combinations. It is probable that such exaggerated proliferative activity is detrimental to the function of the renal tubules, since it is unlikely that the cells engaged in mitosis or the newly-formed polynuclear cells could be normally effective in the performance of their physiological functions. Experiments are now under way, in which the same hormones and hormone combinations are given at a much lower dose level but for a longer period, with the view of obtaining a more gradual and normal increase in renal mass.

Even the most actively growing kidneys, which contained many atypical mitoses, showed no indications of actual tumorous growths. Yet, the atypical proliferation of the epithelial components raises the question whether, under suitable circumstances, renotropic hormones could not induce experimental kidney tumours in the same manner as folliculoid preparations can cause neoplastic proliferations in the organs whose growth they normally stimulate. Here, again, further experiments are necessary to settle the question.

The changes in the renal pelvis were of special interest to us since we apparently missed them in our earlier work concerned with the effect of hormones upon the kidney. Generally speaking, they were most pronounced in those animals in which nephrosclerotic hormones or hormone combinations were given; hence, it is probable that the pelvic changes are associated with the nephrosclerotic, rather than with the renotropic activity of theses preparations. In the absence of any definite information regarding this point, we will discuss the renal pelvis lesions apart from both the renotropic and the nephrosclerotic changes.

Apparently the first stage in the development of these renal pelvis lesions is the formation of an œdema in the adipose tissue surrounding the kidney hilum (see Figs. 14 and 15). The cells of this region lose much of their fat content and are transformed into a more or less irregular, almost myxomatous tissue. Between the cells large accumulations of fluid appear which are readily detectable under the microscope, but visible, in the form of a gelatinous mass, even by mere naked eye inspection. In somewhat more advanced cases granuloma cells appear within the œdematous region. They consist of young fibroblasts, lymphocytes, polymorphonuclear leucocytes (often of the eosinophilic and pseudo-eosinophilic type) as well as mast cells. Only in a few instances did this œdematous granuloma tissue take on a definitely purulent character. In such advanced cases the transitional epithelium of the renal pelvis was often also infiltrated by the inflammatory exudate so that a pyelonephritis resulted. As stated before, the administration of NaCl, as well as the removal of one kidney, sensitizes the experimental animals to the development of pyelonephritis, just as it sensitizes to the nephrosclerotic effect. Without going too far afield in our interpretation of this phenomenon, it is perhaps permissible to raise the question whether some of the so-called "cryptogenic types" of pyelonephritis, seen in clinical medicine, could not be caused by a hormonal imbalance.

The characteristics of experimental nephrosclerosis produced by D.C.A. have been extensively reviewed in our previous publications so that we need not discuss them here in detail. Suffice it to say that, during the early stages, hyalin casts appear within the tubular lumina and the resulting occlusion of the latter causes dilatation of the nephron segments proximal to these casts (see Figs. 10 to 13). In later stages there is round cell infiltration and sclerosis of the stroma between the dilated tubules and eventually hyalinzation of the glomerular capillaries with the formation of a hyalin transudate

in the capsular space. The small renal arterioles, and especially the efferent arterioles of the glomeruli, participate in this process inasmuch as they show hyalin necrosis of the vascular walls and later infiltration by round cells.

Even the large renal arteries in the hilum region are affected inasmuch as the nuclei of the muscular coats undergo degenerative changes and the nuclear chromatin assumes a granular, pyknotic appearance. It is our impression that these changes in the renal blood vessels merely represent a special manifestation of the periarteritis nodosa, so frequently produced by nephrosclerotic compounds in other vascular territories, especially in the mesentery. In those animals of the present experimental series, in which the renal changes were particularly pronounced, periarteritis nodosa was occasionally also seen in other blood vessels; this led to infarcts in the heart and the brain. The changes in the brain were generally limited to the cortical layer and assumed the appearance of an acute apoplexy with necrosis of brain tissue. Animals so affected almost invariably showed clinical manifestations of nervous disturbances such as convulsions, paralysis, etc.

Perusal of the tables reveals that the weight of the heart roughly parallels that of the kidney. It is very probable that this enlargement of the heart is merely a repercussion of the hypertension occasioned by the renal changes. Histologically, the cardiac muscle fibres appear hypertrophic, but degenerative changes were absent, or at least of moderate degree, in those animals in which the kidney showed no sign of sclerosis. Conversely, in the animals in which nephrosclerosis was produced (regardless of the eliciting agent) the cardiac vessels almost invariably showed some sign of periarteritis nodosa.

In addition to this, degenerative and inflammatory lesions were also found among the cardiac muscle fibres and in the stroma of the heart. These latter lesions are rather reminiscent of rheumatic carditis and have repeatedly been described in our earlier experiments on D.C.A. over-dosage; hence, we need not describe them in great detail here. Briefly they may be summarized by saying that within the myocardium small nodules appear, in the centre of which there is generally some hyalinized or necrotic tissue surrounded by a granulomatous zone in which mitotic proliferation, "myocardial histiocytes" (Anitschkow's myocytes or "caterpillar cells") and rather atypical giant cells (not unlike the Aschoff cells) are plentiful. Such granulomatous vegetations are also seen attached to the endocardium of the atria, auricles, ventricles and sometimes on the cardiac valves, especially the mitral valves (see Figs. 16 to 18). It is undeniable that these endocardial and myocardial lesions are very reminiscent of rheumatic carditis and endocarditis, but the relationship of this experimental condition to the acute rheumatic fever of man has not been clarified as yet.

In connection with the present experiments, the important fact is that the degenerative lesions in the heart appeared most frequently and with the greatest intensity in the animals receiving D.C.A. in combination with thyroxin, or other hormone combinations conducive to severe nephrosclerosis.

The adrenal lesions do not require a detailed histological analysis. Suffice it to say that the enlargement of the suprarenal glands was due entirely, or at least prevailingly, to a hypertrophy and hyperplasia of the cortical portion of the gland. There was also marked hyperæmia of the cortex sometimes accompanied by periarteritis nodosa and hæmorrhages into the cortical tissue.

## SUMMARY

In continuation of our work on the etiology and therapy of malignant nephrosclerosis, experiments were performed under diverse conditions in order to examine the effect of various hormones and hormone combinations upon the kidney, the heart and the adrenals of the rat. The principal results of these investigations were the following:

A saline suspension of cattle anterior pituitary tissue causes pronounced hypertrophy and hyperplasia of the renal tubule cells as well as some increase in the diameter of the glomeruli. This renotropic effect is greatly enhanced by the simultaneous administration of thyroxin although the latter hormone possesses only moderate kidney-stimulating properties when given alone. It is noteworthy that the weight of the heart and adrenals is likewise considerably increased under the influence of this combined treatment with thyroxin and the hypophyseal preparations; the increase being of such magnitude that we may well speak of potentiation rather than of mere summation.

The nephrosclerotic potency of D.C.A. is also enhanced by simultaneous thyroxin administra-

tion. Animals treated with D.C.A. developed nephrosclerosis, œdema or inflammatory changes in the fat tissue surrounding the renal pelvis, degenerative and inflammatory changes in the heart muscle and endocardium (these resemble the lesions characteristic of rheumatic carditis) as well as vascular lesions of the periarteritis nodosa type. All these overdosage effects of D.C.A. are considerably enhanced by simultaneous treatment with thyroxin. Combined administration of D.C.A. and the hypophyseal preparations likewise resulted in over-dosage symptoms, similar to those described above.

In confirmation of our previous experiments, we found that methyl-testosterone exhibits a renotropic action, inasmuch as it induces hypertrophy and hyperplasia of the renal tubules. It does not noticeably increase the diameter of the glomeruli. Unlike the renotropic anterior lobe preparation, the renotropic action of methyl-testosterone is not potentiated by simultaneous administration of thyroxin, but the kidney-stimulating effects of the two hormones are apparently merely summated.

Combined administration of methyl-testosterone and the hypophyseal preparation, likewise resulted only in a summation and not in true potentiation of their renotropic effect.

Removal of one kidney and the administration of NaCl in high doses greatly sensitizes the rat to the nephrosclerotic effect of various hormones and hormone combinations. Such sensitization also increases the incidence of cardiac lesions, periarteritis nodosa and œdema of the renal pelvis. On the other hand, the true renotropic (kidney growth-stimulating) effect of various hormones and hormone combinations was not significantly augmented by unilateral nephrectomy and a high NaCl diet; it appears as though after unilateral nephrectomy the growth stimulating effect of the hormone treatment were merely concentrated on the remaining kidney instead of being equally distributed between the two organs.

Under the influence of very potent renotropic hormone combinations (*e.g.*, hypophyseal preparation plus thyroxin) innumerable mitotic divisions (some atypical) were noticed in the lining cells of the renal tubules.

The adrenotropic effect of the anterior lobe extract was enormously enhanced by simultaneous treatment with thyroxin.

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## THE PRESENT STATUS OF THE INTERNAL FIXATION OF FRACTURES*

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WITHOUT benefit of historical record, there is little doubt that the first major surgery attempted by our most primitive ancestor was the setting of a fracture. He was content with the closed method, as were his increasingly learned progeny, for some thousands of years.

When M. Lapeyoden, in 1795, emboldened by operative success in other fields, began wiring broken bones, his septic disasters discouraged emulation. But with the coming of Lister and his new era, as one after another of the body fastnesses became surgical hunting grounds, the first open reduction phase in fracture therapy grew to a rapid maturity under the brilliant, driving efforts of its protagonist, Arbuthnot Lane.

Lane was a surgical architect. He saw the skeleton as a bony framework that must

* Written from a talk given for the Dalhousie Refresher Course, 1942-43.