detector is placed at the boundary of the intact and crushed region, then but one peak will be observed.

Details of the apparatus used for the detection of these electrical lines of force are given in the above-mentioned report; however, the general features are shown in Fig. 1.

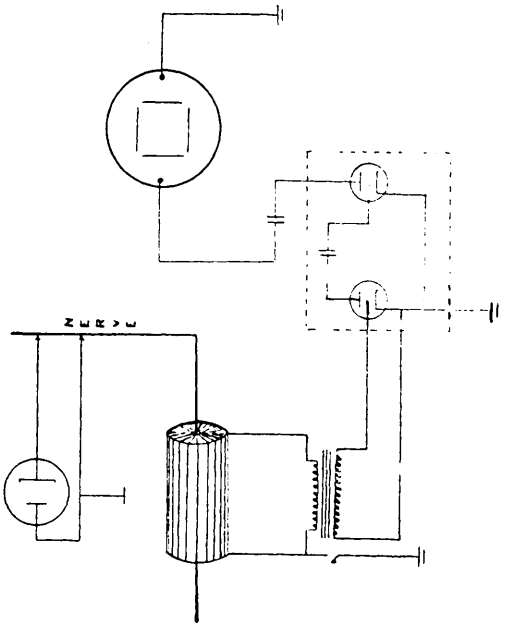
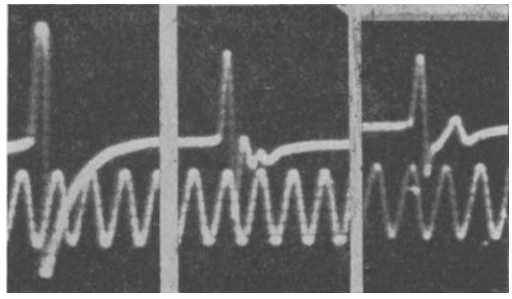


FIG. 1.

That the disturbances indicated by the recording oscillograph were in the nature of induced electrical charges on the detecting coil and not an induced e.m.f. is shown by the fact that they disappeared when the primary coil of the matching transformer was grounded. (*Cf.* Fig. 1.)

The frog's sciatic was used. The results are indicated in Fig. 2. A gives the stimulus artifact (*ca.* 30 m V); B shows the result obtained with an intact nerve, and reveals the 2 peaks; C is the result obtained when the detector is located on the boundary of the intact and crushed portion.



A B C

FIG. 2.

A. Stimulus artifact obtained with dead nerve.
B. The two peaks obtained with intact nerve.
C. Single peak obtained when detector is located on boundary of crushed and intact portion of the nerve. Time line indicates 500 cycles.

It was possible to duplicate these results when the nerve and the detector were both immersed in tap water, mineral oil, and Ringer's solution. In the case of Ringer's, it was possible to demonstrate increased velocity of the nerve impulse as being of the order of value calculated by Hodgkin[2] by other experimental means.

[2] Hodgkin, A. L., *J. Physiol.*, 1939, **94**, 510.

14082

Role of Sodium Chloride in Production of Nephrosclerosis by Steroids.

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Previous investigations[1] have shown that desoxycorticosterone acetate (D.C.A.) causes nephrosclerosis and generalized tissue edema in the chick. The condition is usually accompanied by cardiovascular changes similar to those seen in human hypertensive heart disease. In the present communication we should like to discuss a series of experiments indicat-

[1] Selye, Hans, *Canad. Med. Assn. J.*, 1942, **47**, 515.

ing that high doses of NaCl added to the drinking water of chicks may in themselves suffice to elicit all these changes. Such salt treatment causes death due to the development of a condition similar to that of the naturally-occurring Bright's disease[2] or "pullet disease"[3] of the fowl. On the other hand, D.C.A. elicits these changes even in birds kept on a normal salt intake and potentiates the effect of subthreshold doses of NaCl.

In the first experimental series 6 male and 6 female 2-day-old single-combed White Leghorn chicks weighing 35-38 g (average 35 g) were given 2% NaCl solution instead of drinking water. All of these birds died with generalized tissue edema and cardiac dilatation within 3 days. The experiment was then repeated on 6 male and 6 female 3-week-old Barred Rock chicks which received 0.9% NaCl instead of drinking water. Eight of these died within 20 days and the remaining 4 were killed at the end of this time. All 12 animals showed generalized tissue edema, fluid accumulations in the serous cavities and cardiac dilatation. Their kidneys were enlarged and histologically characterized by glomerular sclerosis, tubular hypertrophy and numerous casts. This is essentially the same picture as that previously obtained with D.C.A. in chicks kept on a normal salt intake.[1]

Additional experiments were then performed in order to establish whether sub-threshold doses of NaCl—which in themselves are not toxic—could sensitize the bird to the above-mentioned actions of D.C.A. We felt that if this were so we would not only gain further information concerning the pathogenesis of these lesions, but would also acquire a highly sensitive test object, suitable for the estimation of nephrotoxic potency in steroids other than D.C.A. One hundred seventy seven-day old, single-combed White Leghorn pullets were subdivided into 17 groups of 10 birds each. The average initial body weight in each group was 44 g (range 38-52 g). At such an early age it is difficult to tell the sex of live pullets but autopsy at the end of the experiment revealed that the number of males and females was approximately the same in the various groups. All the steroids assayed were administered subcutaneously in the form of finely ground crystals suspended in water. Two mg in 0.2 cc of distilled water were given twice daily. This injection treatment was combined with the administration of an 0.2% NaCl solution instead of ordinary drinking water. This weak solution does not cause any nephrosclerosis when given by itself as shown by previous experiments and by the group receiving it in combination with inactive steroids (*e.g.* cholesterol). However, we anticipated that it would sensitize the birds to nephrotoxic steroids. In order to show this sensitization, one group of D.C.A. treated birds received the above NaCl solution while another received tap water for comparison. It has been claimed that potassium salts antagonize many actions of sodium and that certain D.C.A. overdosage symptoms are counteracted by potassium ions.[4,5] Hence in one group we administered the above NaCl solution with KCl added in an equimolecular concentration. A third group received only the KCl solution of this same concentration (0.25%). All animals were killed 10 days after initiation of the treatment and the prominent findings are summarized in Table I. The common names of the various steroids are given in italic letters and the systematic chemical names are likewise mentioned as well as the melting point of the specimen which we used. This will help to identify the compounds in a definite manner and to indicate the degree of purity of our preparations.

Perusal of the table indicates that an NaCl solution which in itself is not toxic to the chick becomes toxic if given in conjunction with comparatively small doses of D.C.A. The toxicity of this corticoid—as evidenced by the degree of edema and the number of deaths caused—is about twice as marked in

2 Weaver, C. H., *Proc. 14th Ann. Conf. Lab. Workers in Pullorum Disease,* Ottawa, Canada, May, 1941.

3 Jungherr, Erwin, and Levine, Jacob M., *Am. J. Vet. Res.*, 1941, **2**, 261.

4 Pudenz, Robert H., McIntosh, John F., and McEachern, Donald, *J. Am. Med. Assn.*, 1938, **111**, 2253.

5 Kuhlmann, Daniel, Ragan, C., Ferrebee, J. W., Atchley, Dana W., and Loeb, Robert F., *Science,* 1939, **90**, 496.

TABLE I.

Steroid treatment	M.P.°C	Salt treatment	No. of deaths before 10th day	Degree of edema
Desoxycorticosterone acetate 17-ethyl-Δ^4-androstene-3,20-dione-21-ol acetate	152	tap water	4	++
Desoxycorticosterone acetate	152	NaCl	8	++++
Desoxycorticosterone acetate	152	KCl	3	++
Desoxycorticosterone acetate	152	NaCl + KCl	6	++++
Progesterone 17-ethyl-Δ^4-androstene-3,20-dione	128	NaCl	4	++
Pregnenolone 17-ethyl-androstene-3(β)-ol-20-one	186	"	0	0
Pregnanedione 17-ethyl-etiocholane-3,20-dione	121	"	0	0
Δ^{16}-*pregnenedione* 17-ethyl-Δ^{16}-etiocholane-3,20-dione	190	"	0	0
Ethinyl testosterone 17-ethinyl-Δ^4-androstene-3-one-17(α)-ol	265-268	"	0	0
Testosterone Δ^4-androstene-3-one-17(α)-ol	154	"	0	0
Androstenediol Δ^5-androstene-3(β),17(α)-diol	182-183	"	0	0
Dehydro-iso-androsterone Δ^5-androstene-3(β)-ol-17-one	137	"	0	0
Androstenedione Δ^4-androstene-3-17-dione	169-171	"	0	0
Estradiol $\Delta^{1,3,5}$-estratriene-3,17(α)-diol	176-177	"	0	0
*Adrenal cortical extract**	—	"	0	0
Nor-cholestenolone 17-*iso*-heptyl-Δ^5-androstene-3(β)-ol-25-one	127-128	"	0	0
Cholesterol 17-*iso*-octyl-Δ^5-androstene-3(β)-ol	149	"	0	0

*Prepared by Professor E. C. Kendall. 1 cc represents 75 g of fresh adrenal glands and contains approximately 40 units as defined by Selye and Schenker.[6] 0.25 cc were given twice daily subcutaneously.

chicks receiving the NaCl solution than in those given tap water. Equimolecular doses of KCl do not appear to exhibit this potentiating action of NaCl, nor can they inhibit the effect of the latter. Progesterone, which like D.C.A. exhibits definite corticoid actions, is the only other steroid among those investigated, which is toxic to NaCl sensitized chicks. It is of interest in this connection that progesterone also shares with D.C.A. the diuretic effect of the latter.[7] Further experiments will have to show whether other corticoids, especially the carbohydrate active ones oxygenated at C_{11}, exhibit this effect. It is noteworthy, however, that the adrenal extract, which is rich in such corticoids, caused no edema at least in the doses used.

The histologically detectable renal changes were not as pronounced as in the more chronic experiments mentioned above,[1] however, they were qualitatively of the same type both in the D.C.A. and in the progesterone series (see Fig. 1-3). The convoluted tubules showed signs of hypertrophy and sometimes cloudy swelling with desquamation. Many of them contained hyaline casts. The glomeruli were enlarged and in many cases the cells of the parietal lamina of Bowman's capsule were unusually high. There also was some hyalinization of glomerular capillaries and proliferation of epithelioid cells in the region of the glomerular capsule. No histological changes were detectable in the kidneys of the chicks receiving compounds other than D.C.A. or progesterone.

Although the chick is by far the most sensitive test animal for this particular effect of the steroids it should be mentioned that essen-

6 Selye, Hans, and Schenker, Victor, PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 518.

7 Selye, Hans, and Bassett, Lucy, PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 502.

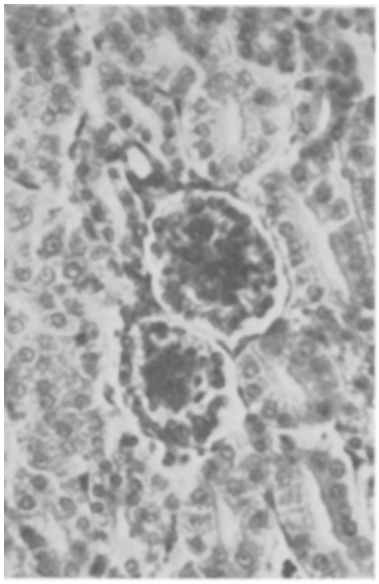

FIG. 1.
Normal kidney of chick receiving NaCl and cholesterol.

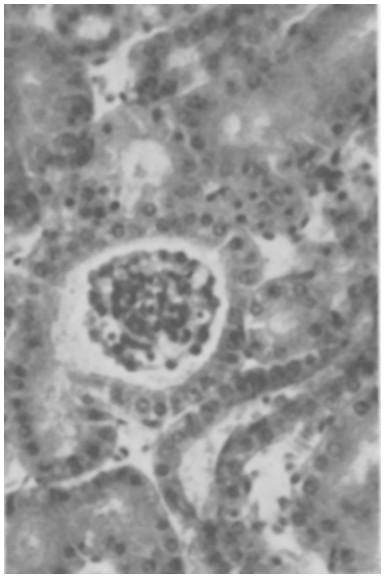

FIG. 2.
Kidney of chick receiving NaCl and D.C.A. Note hypertrophy of convoluted tubules and of parietal lamina of Bowman's capsule.

tially similar renal lesions have also been produced in the rat, dog and monkey.[8] Hence

[8] Selye, Hans, and Hall, C. E., *Arch. Path.*, in press.

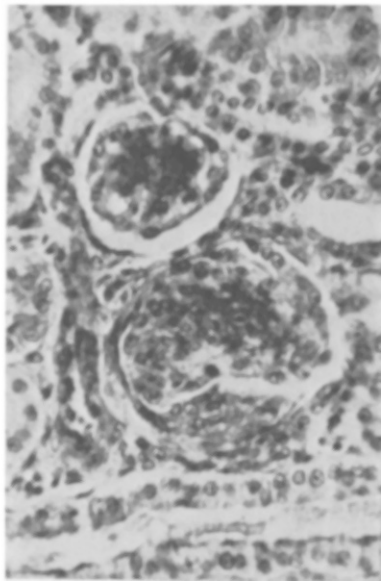

FIG. 3.
Kidney of chick receiving NaCl and progesterone. Note "epithelioid crescent" in one of the renal corpuscles.

it is not impossible that disturbances in water metabolism and certain types of nephrosclerosis may occur even in man as a result of excessive production of D.C.A., progesterone or other pharmacologically related steroids.

Conclusions. Administration of concentrated NaCl solutions causes generalized tissue edema and nephrosclerosis in young chicks. Desoxycorticosterone acetate (D.C.A.) exerts a similar effect even on a normal salt intake and sensitizes chicks to the action of subthreshold concentrations of NaCl. Among a number of steroid preparations tested for this effect only progesterone was found to possess this activity and even this compound was not quite as active as D.C.A.

The expenses of this investigation were defrayed through a grant received from the DesBergers-Bismol Laboratories of Montreal, Canada. We are greatly indebted to Dr. Erwin Schwenk of the Schering Corporation of Bloomfield, N.J., for all steroid compounds except the pregnanedione which was supplied by Professor R. D. H. Heard and the Δ^{16}-pregnenedione donated by Dr. O. Kamm of Parke, Davis and Company. Our thanks are also due to Dr. S. S. Munro of the Canadian Department of Agriculture who furnished the chicks for these experiments.