# ALDOSTERONE AND ALDOSTERONISM

E. J. ROSS
M.D., F.R.C.P.

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E. J. Ross

*Professor of Endocrinology, University College Hospital Medical School, London*


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# ALDOSTERONE AND ALDOSTERONISM

The discoverers of aldosterone (*see* p. 3)

*Left to right:* T. Reichstein, S. A. Simpson (now Mrs. J. F. Tait), J. F. Tait, A. Wettstein, R. Neher and J. von Euw.

# Preface

A previous monograph on aldosterone and aldosteronism, *Aldosterone in Clinical and Experimental Medicine,* which was published in 1959, is now so out of date as to demand the writing of a new book. In this, an attempt has been made to survey world literature, to present an account of the development of our knowledge of this hormone and to evaluate current ideas on its biological role and its part in the causation of disease.

The literature of aldosterone is now so voluminous that some selection has been inevitable. This steroid first achieved the status of an individual entry in the *Current List of Medical Literature* and its successor, the *Cumulative Index Medicus,* in 1957. The number of entries yearly have been as follows:

| | | | | | |
|---|---|---|---|---|---|
| 1957 | 99 | 1963 | 211 | 1969 | 205 |
| 1958 | 114 | 1964 | 353 | 1970 | 187 |
| 1959 | 138 | 1965 | 283 | 1971 | 208 |
| 1960 | 194 | 1966 | 129 | 1972 | 207 |
| 1961 | 250 | 1967 | 165 | 1973 | 190 |
| 1962 | 313 | 1968 | 210 | | |

Quotations from the literature have been made from the contributions that have advanced our understanding of the biochemistry and physiology of aldosterone and its role in homeostasis, as well as its contribution to disease. An attempt has been made to read all published work on this and related topics up to mid-1974 as widely as possible, but the writer apologises to those collegues whose work has been overlooked and assures them that this has been solely due to neglect on his part, since no work has been quoted that has not been read and many references must have escaped his scrutiny.

The author also apologises to his colleagues whose identity is concealed by the use of "et al" in references, but the use of this abbreviation was dictated by considerations of space as about 3,500 references have been quoted.

The final presentation for press has been made infinitely less tedious by the painstaking care and industry of Mr. G. J. Hooton of Messrs. Lloyd-Luke (Medical Books) Ltd. Miss E. Tinlin has been plagued by numerous retypings of the original manuscript. I am very grateful to both of them for their tolerance.

I wish to acknowledge my indebtedness to the following for permission to reproduce certain illustrations and other data: *Acta Endocrinologica* for Table IV; *American Association for the Advancement of Science* for Fig. 16 and Table IX; *American Heart Association* for Fig. 5: *American Journal of Medicine* for Fig. 21; *Blackwell Medical Publications* for Tables XV and XVI; *British Medical Journal* for Figs. 13, 17, 19, 20 and 27; *Clinical Pharmacology and Therapeutics* for Fig. 18; *Clinical Science* for Figs. 9 and 25 and for Table XVIII; *Journal of Clinical*

*Investigation* for Figs. 8*a*, 10 and 15; *Journal of Endocrinology* for Table VIII; *Journal of Laboratory and Clinical Medicine* for Table XIV; *Lancet* for Fig. 14; *Medicine, Baltimore* for Fig. 11; *Proceedings of the Royal Society of Medicine* for Table VII; *University of Michigan Medical Bulletin* and *Journal of Steroid Biochemistry* for Table XII. The Frontispiece is reproduced by courtesy of Professor A. Wettstein and by permission of the *Journal of Steroid Biochemistry*.

*August*, 1974 E. J. R.

# Nomenclature

Steroids have been named according to the rules laid down by the International Union of Pure and Applied Chemistry Commission on the Nomenclature of Organic Chemistry and the International Union of Biology Commission on Biochemical Nomenclature. The following trivial names have been used:–

| | |
|---|---|
| aldosterone (electrocortin) | 11-18 hemiacetal of 11β,21-dihydroxy-3,20-dioxo-4-pregnene,18-al |
| corticosterone (compound B) | 11β,21-dihydroxy-4-pregnene-3,20-dione |
| 11-deoxycorticosterone (deoxycortone, cortexone, DOC) | 21-hydroxy-4-pregnene-3,20-dione |
| cortexolone (17-hydroxy,11-deoxycorticosterone, 11-deoxycortisol, substance S) | 17α,21-dihydroxy-4-pregnene-3,20-dione |
| 11-dehydrocorticosterone (compound A) | 21-hydroxy-4-pregnene-3,11,20-trione |
| 17-hydroxy,11-dehydrocorticosterone (cortisone compound E) | 17α,21-dihydroxy-4-pregnene-3,11,20-trione |
| 17-hydroxycorticosterone (cortisol,hydrocortisone, compound F) | 11β,17α,21-trihydroxy-4-pregnene-3,20-dione |
| 9α-fluorocortisol (9α-fluorohydrocortisone, fluorocortisone) | 9α-fluoro,11β,17α,21-trihydroxy-4-pregnene-3,20-dione |
| dexamethasone | 9α fluoro,11β,17α,21-trihydroxy,16α-methyl-1,4-pregnene-3,20-dione |
| prednisone | 17α,21-dihydroxy-1,4-pregnene-3,11,20-dione |
| prednisolone | 11β,17α,21-trihydroxy-1,4-pregnene-3,20-dione |
| pregnenolone | 3β-hydroxy-5-pregnene-20-one |
| progesterone | 4-pregnene-3,20-dione |
| cholesterol | 5-cholestene-3-β-ol |

*Note:*– Equivalent concentrations of Aldosterone $10^{-4}$ μg/ml = $2.77 \times 10^{-7}$ mol/l.

# Abbreviations

| | |
|---|---|
| A | 11-dehydrocorticosterone |
| ACE | adrenal cortical extract |
| ACTH | adenocorticotrophic hormone, corticotrophin |
| ADH | antidiuretic hormone |
| ADP | adenosine diphosphate |
| aldo | aldosterone |
| AMP | adenosine 5′-monophosphate |
| ASH | aldosterone stimulating hormone |
| ASR | aldosterone secretion rate |
| ATP | adenosine 5′-triphosphate |
| B | corticosterone |
| Ca | calcium |
| Co-A | coenzyme A |
| cyclic-AMP (3′,5′-AMP) | 3′5′-adenylmonophosphate |
| DCA | 11-deoxycorticosterone acetate |
| DNA | deoxypentose nucleic acid |
| DOC | 11-deoxycorticosterone |
| E | cortisone |
| F | cortisol |
| 9α-FF | 9α-fluorocortisol |
| FAD | flavine-adenine dinucleotide |
| h | hour(s) |
| K | potassium |
| $K_e$ | total exchangeable potassium |
| min | minute(s) |
| Na | sodium |
| $Na_e$ | total exchangeable sodium |
| NAD | nicotinamide-adenine dinucleotide (without indication of state of oxidation) |
| $NAD^+$ | oxidised nicotinamide-adenine dinucleotide |
| NADH | reduced (dihydro) nicotinamide-adenine dinucleotide |
| $NADP^+$ | oxidised nicotinamide-adenine dinucleotide phosphate |
| NADPH | reduced nicotinamide-adenine dinucleotide phosphate |
| PRA | plasma renin activity |
| RNA | ribonucleic acid<br>m = messenger s = soluble<br>n = nuclear t = transfer |

# Contents

# 1 The Discovery of Aldosterone

Clinical interest in the adrenal gland dates back over a period of more than 100 years to Addison's observation that destruction of the "suprarenal capsules" by tuberculosis, cancer or "idiopathic atrophy" rapidly led to death (Addison, 1855). Recognition of the fatal consequences of the experimental removal of the adrenal glands in animals soon followed (Brown-Séquard, 1856). Attempts to isolate the life-preserving constituent of these glands were fruitless until 1896, when Sir William Osler reported striking clinical improvement in a patient with Addison's disease to whom a glycerine extract of fresh hog adrenal glands had been given by mouth. The isolation of adrenaline from adrenal medullary extracts led to the anticipation that this would maintain life in the adrenalectomised animal and it was only after unexpected failure to do so that it was recognised that the vital fraction resided in the cortex of the gland (Biedl, 1913).

The observation that adrenalectomy in animals was followed by a fall in serum sodium concentration and rise in serum potassium concentration (Baumann and Kurland, 1927) drew attention to the role of the adrenal glands in the regulation of the concentrations of these important cations in the extracellular fluid. This observation was complemented by the finding that the effects of total adrenalectomy in animals could be ameliorated by the administration of sodium chloride (Banting and Gairns, 1926; Marine and Baumann, 1927; Rogoff and Stewart, 1927) and that death was hastened when the animals were given potassium salts. The clinical counterpart of these experimental results in animals was the prolongation of survival time in patients with Addison's disease when they were given sodium chloride by mouth and their sensitivity to overdosage with potassium salts (Harrop, 1933; Loeb, 1933; Loeb et al. 1935; Kepler, 1935).

In the 1920s, three groups independently reported the preparation of adrenocortical extracts which were claimed to prolong the life of adrenalectomised animals. Little information is available about the extract prepared by Goldzieher (1929). The significance of the observations of Stewart and Rogoff (1929) has been doubted on statistical grounds (Hartman and Brownell, 1949). Rogoff later prepared a glycerine extract which was effective when given by mouth to a patient with Addison's disease (Rogoff, 1932). The extract prepared by Hartman et al. (1927) doubled the survival time of adrenalectomised cats. Another extract prepared a little later was alleged to keep adrenalectomised cats alive "indefinitely" (Hartman, 1930) and was also successful clinically in terminating an Addisonian crisis. The aqueous extract prepared by Swingle and Pfiffner (1931) was likewise effective in patients with Addison's disease and the acetone-ethylene dichloride extraction procedure of Cartland and Kuizenga (1936, 1939) was developed commercially in the manufacture of "adrenal cortical extract" (ACE) from hog adrenals.

The rapid advance in knowledge of the chemistry of steroids during the 1930s

led to the isolation of numerous crystalline compounds from adrenal cortical extracts. Some were found to possess significant biological activity: these included corticosterone (Compound B of Kendall), cortisone (Compound E of Kendall), cortisol (Compound F of Kendall), 11-dehydrocorticosterone (Compound A of Wintersteiner and Pfiffner), 11-deoxycortisol (Substance S of Reichstein), adrenosterone, oestrone and progesterone.

When all the known crystalline steroids had been removed from adrenal cortical extract there remained a non-crystalline residue, the "amorphous fraction" which contained at least 50 per cent of the sodium-retaining activity of the original extract. Many attempts were made to extract sodium-retaining compounds from this amorphous fraction. Grollman (1939) claimed to have isolated a crystalline material that was a hundred times more active than the synthetic mineralocorticoid 11-deoxycorticosterone but was unable to identify the chemical nature of this material. A concentrate possessing high sodium-retaining activity was also prepared by Hartman and Spoor (1940), which maintained adrenalectomised dogs in good condition for a long period. The substance responsible for this effect on the renal handling of sodium was named the "sodium factor" (Hartman et al. 1939). It possessed considerably more sodium-retaining activity than 11-deoxycorticosterone, on a weight basis. It could not have been aldosterone as it was not soluble in ethyl alcohol or chloroform.

The biological activity of adrenal cortical extract with respect to carbohydrate and protein metabolism could be accounted for adequately by its content of cortisol and other crystalline steroids. Some investigators (e.g. Fourman et al. 1950; Verzar, 1950) thought that the sodium-retaining activity of adrenal cortical extract could also be accounted for by its content of cortisol, but this obviously could not be so as the amorphous fraction possessed sodium-retaining activity equal to that of the synthetic steroid 11-deoxycorticosterone (DOC) which is many times more active than cortisol in this respect. Deoxycorticosterone, however, had not at that time been detected in biological fluids. Many had come to doubt whether a separate mineralocorticoid really existed, although a substance with these properties was required by Selye's postulate of the "stress reaction", which advocated the functional separation of adrenal steroids into "glucocorticoids" and "mineralocorticoids" (Selye, 1946). In reality, the crystalline substances isolated by the chemists from adrenal cortical extracts have, to greater or lesser degree, mixed glucocorticoid and mineralocorticoid activity, so that it was not necessary to postulate the existence of a new steroid with purely mineralocorticoid activity. It did seem, however, that a substance as potent as, or even more potent in sodium-retaining activity than, 11-deoxycorticosterone must be secreted by the adrenal gland.

From 1950 onwards, evidence suggesting the existence of such a naturally occurring substance began to appear. Progress in its recognition was consequent upon the development of techniques of biological assay based on alteration of the renal excretion of sodium and potassium in suitably prepared adrenalectomised rats. In particular, Simpson and Tait (1952) evolved a method of assay based on alteration of the ratio of radioactive sodium ($^{24}Na$) and radioactive potassium ($^{42}K$) excreted in the urine; this greatly simplified the measurement of sodium and potassium in days when flame photometry was in its infancy and flame photometers scarce.

Using this technique, Tait et al. (1952) at the Middlesex Hospital Medical

School, London, examined "Eucortone", a commercially available adrenal cortical extract prepared from beef adrenal glands. They found that its activity in the bioassay, on a weight basis, exceeded that of all known naturally occurring adrenal hormones and resembled that of 11-deoxycorticosterone. This group of workers then proceeded to apply the newly developed technique of paper chromatography to the separation of the steroid compounds present in adrenal cortical extract, in conjunction with the biological assay mentioned above. Using the toluene-propyleneglycol chromatographic system of Burton et al. (1951), 87 per cent of the original sodium-retaining activity of adrenal cortical extract was found in the fraction with running properties identical with the cortisone reference standard. The active material could not be identical with cortisone, however, since its biological activity was some 50 times that of cortisone, weight for weight. Nor was it 11-deoxycorticosterone, since this substance ran much faster than cortisone in the chromatographic system used. It was therefore concluded that the sodium-retaining activity found on bioassay was due to the presence of an unknown substance with chromatographic mobility in this system which was identical with that of cortisone.

Simpson et al. (1952a) then proceeded to extract and separate by paper chromatography the steroids in adrenal vein blood obtained from a rhesus monkey and a dog. The eluate from the chromatogram which ran with the speed of cortisone possessed biological activity equivalent to 15 $\mu$g of 11-deoxycorticosterone, yet only weighed 2 $\mu$g. In the extract of dog's blood, the cortisone spot on the chromatogram had a biological activity equal to 56 times the known sodium-retaining action of cortisone.

These findings confirmed the suspicion that there was present in adrenal gland extract and adrenal venous blood a substance with an effect on the renal reabsorption of sodium and potassium which was much greater than was possessed by any substance hitherto described. This compound was provisionally called "electrocortin" (Simpson and Tait, 1953). Separation of "electrocortin" from cortisone was achieved chromatographically by re-running the mixture in the benzene-water-methanol system of Bush (1952). Preliminary experiments showed that it possessed a perhydrocyclopentenophenanthrene nucleus with an unsaturated bond between carbon atoms 4 and 5, and a ketone group on atom 3 in ring A and also an $\alpha$-ketol side chain attached to ring D in the beta position, as judged by its behaviour in the blue tetrazolium and soda fluorescence tests (Simpson and Tait, 1953). The substance could be acetylated, but the acetylated compound had no biological activity; this was regenerated when the acetate was hydrolysed.

Further characterisation of "electrocortin" necessitated large-scale extraction of beef adrenocortical extract provided by the Organon Laboratories. This was achieved by column chromatography in a collaborative effort in 1952 between Mrs. Simpson and Dr. Tait of the Middlesex Hospital Medical School, London, Drs. Wettstein, Neher and Schindler and Mr. von Euw of the Research Laboratories of Ciba Ltd., Basel and Professor Reichstein of the Department of Organic Chemistry of the University of Basel (1954). Professor Reichstein (1972) and Professor Wettstein (1972) have recently reminisced about this collaboration that saw the birth of aldosterone. The frontispiece of this book records a lighter moment in this joint endeavour, taken in Basel "towards midnight, between trains, at a bar near the railroad station" (Wettstein, 1972) and is reproduced by the courtesy of Professor Wettstein. The yield of "electrocortin" was 40-95 $\mu$g per kilogram of fresh beef

adrenal glands. A similar crystalline material with strong sodium-retaining properties was obtained shortly afterwards by Mattox et al. (1953b), of the Mayo Clinic and by Knauff et al. (1953) of Princeton University. Mattox and Mason (1956) later obtained a yield of 48 $\mu$g per kilogram of been adrenal gland. Ham et al. (1955) of the Merck Company were also successful in isolating aldosterone from beef adrenal glands at an early date.

The isolation of 56 mg of aldosterone from 1000 kg of beef adrenal glands enabled its molecular constitution to be evaluated. This was achieved by Simpson et al. (1954a). The various steps in this process are described by them and by Wettstein and Anner (1954) and will not be recapitulated here. "Electrocortin" proved to be the 18-aldehyde of corticosterone (see Fig. 1). It was the first naturally occurring biologically active steroid aldehyde to be described and accordingly was given the definitive name "aldosterone" (Simpson et al. 1954a). It has since been successfully synthesised and its chemical structure confirmed. This is discussed further in the next chapter. In view of initial difficulties with its synthesis it reflects great credit on the organic chemist and pharmaceutical industry that aldosterone is currently available commercially (as Aldocorten®, Ciba) at a cost of about £0.50 per milligram.

Meanwhile two other lines of approach had been converging, culminating in the recognition of the presence of a potent sodium-retaining substance in urine and in adrenal vein blood. Grollman and Firor (1933) had found that extracts of human urine prolonged the life of adrenalectomised cats. Luetscher and Deming (1950), using sodium retention in the adrenalectomised rat preloaded with saline as a method of assay and deoxycorticosterone acetate as reference standard, found that sodium-retaining activity was present in chloroform extracts of acidified urine obtained from patients suffering from disorders characterised by oedema. They noted that the elevated excretion of sodium-retaining factor declined when a diuresis occurred either spontaneously (Luetscher and Deming, 1950) or was induced by the administration of cortisone or corticotrophin (Luetscher et al. 1951). Increased amounts of sodium-retaining factor in chloroform extracts of acidified urine from patients with nephrosis were also found by Singer and Venning (1953) and by McCall and Singer (1953); again, the latter also noted that disappearance of sodium-retaining activity accompanied a spontaneous diuresis. Only slight activity, equivalent to the excretion of 40-80 $\mu$g of deoxycorticosterone acetate per 24 h, was found in the urine of normal adults on a normal sodium intake, and greatly increased activity in the urine of a pateint with cirrhosis (Chart and Shipley, 1953). When the dietary sodium intake by normal subjects was restricted, an increased quantity of sodium-retaining factor was excreted compared with the amount excreted when the sodium intake was within the normal range (Luetscher and Axelrad, 1954a). Normal values were also found in the urine of patients with hypopituitarism (Luetscher and Axelrad, 1954b) whereas in patients with Addison's disease, sodium-retaining activity was not detectable in the urine (Luetscher, 1956).

Attempts were then made to isolate the substance responsible for this biological effect. Paper chromatography of the chloroform extract of acidified urine by the toluene-propyleneglycol system of Zaffaroni et al. (1950) showed that the sodium-retaining activity of urine extracts moved on the paper at a rate similar to that of cortisone, but was not cortisone since this steroid had no sodium-retaining activity when assayed in comparable amounts (Simpson and Tait, 1950). Further

paper chromatographic separation of the material eluted from the cortisone band on the Zaffaroni chromatogram showed that the "sodium-retaining factor" isolated from the urine of patients with the nephrotic syndrome (Luetscher et al. 1955) and cirrhosis (Wolff et al. 1956b) was identical with aldosterone.

Aldosterone was also detected in adrenal vein blood. An indication that a potent sodium-retaining factor might be present in adrenal vein blood was provided by Spencer in 1950. He found sodium-retaining activity equivalent to 4 $\mu$g of deoxycorticosterone acetate per millilitre of dog adrenal vein blood, when assayed by its effect on the sodium excretion of adrenalectomised mice. No activity was detected in blood from the carotid artery of the same animal. Sodium-retaining activity, later proved to be due to aldosterone, was then found by Simpson et al. (1952a) in adrenal vein blood from a dog and in blood perfused through the isolated adrenal gland of a monkey. It was this observation that led to an examination of beef adrenal extract for mineralocorticoid activity (Grundy and Simpson, 1952) and to its subsequent isolation, detailed above.

Aldosterone has subsequently been demonstrated in the peripheral blood of fishes, reptiles, amphibians and birds as well as in animals and man.

# 2 Chemical Properties of Aldosterone

### Constitution

Aldosterone is the 18-aldehyde of corticosterone, i.e. is 11-$\beta$,21-dihydroxy-3,20-dioxo-4-pregnene,18-al. To date, it is unique in being the only known naturally occurring, biologically active steroid aldehyde.

### Isomerism

The naturally occurring steroid is the *d*(+)-enantiomer. *d*-Aldosterone exists in two isomeric forms, as the 18-aldehyde of corticosterone and as the 11 → 18 hemiacetal (Fig. 1). The latter is the more stable form at the hydrogen ion concentration of blood; aldosterone presumably exists in this form in plasma since its products of metabolism retain this ring structure.

A third isomer, the 20-hemiketal, also exists (Ham et al. 1955) and has been isolated from perfused adrenal glands (Kraulis and Birmingham, 1964), but it is not yet known whether it possesses biological activity.

The isomer 17-iso-aldosterone, which has the side chain attached to C-17 in the alpha-position, is formed from the naturally occurring 17 beta-aldosterone under alkaline conditions and becomes a nuisance as a contaminant when aldosterone is stored in solution, e.g. in ampoules of tritiated aldosterone used for measurement of secretion rates. For this reason, the purity of aldosterone solutions should always be checked chromatographically before use in experimental investigations.

### Crystallisation

Aldosterone crystallises from acetone-ether or acetone-water solutions as a monohydrate, with a double melting point, first at 108-112° C. The anhydrous substance melts at 155-160° C. These solutions contain other crystals which become opaque at 115-135° C and melt at 164° C (Simpson et al. 1954b). Crystallisation from dry ether yields a product which melts at 163-164° C (Mattox et al. 1953b).

### Synthesis

The first partial synthesis of *dl*-aldosterone was successfully achieved by Schmidlin et al. (1955) from a tricyclic phenanthrene compound by a process involving more than 20 stages. The racemic compound was resolved by microbiological methods into the *d*-isomer by Vischer et al. (1956). Simpler partial syntheses have been reported by Lardon et al. (1957) and Heusler et al. (1960) and by Nagata et al. (1962). Total synthesis has also been achieved by Szpilfolgel et al. (1956 and 1958), van der Berg et al. (1958), Barton and Beaton (1961), Johnson et al. (1963) and Velluz et al. (1960). Readers are referred to these papers for further particulars and to Wolff et al. (1960, 1963).

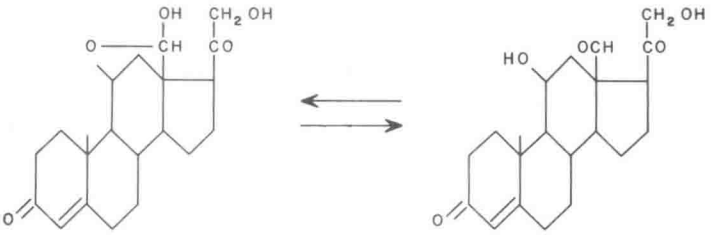


FIG. 1. Formula of aldosterone. Hydroxy-aldehyde form on the right and cyclohemiacetal form on the left.

### Physiochemical Properties

Various chemical and physical constants are given in Table 1. Aldosterone has a 4-dehydro,3-oxo configuration in ring A, as have other biologically active steroids, and so absorbs ultraviolet light, a property utilised in its localisation in paper and thin-layer chromatography.

TABLE I

*Chemical and physical constants of aldosterone*

| | |
|---|---|
| Empirical formula | $C_{21}H_{28}O_5$ |
| Elemental composition | carbon = 69.9 per cent<br>hydrogen = 7.83 per cent<br>oxygen = 22.2 per cent |
| Molecular weight | 360.44 |
| Melting point | 164°C (anhydrous form)<br>108-112°C (hydrated crystals) |
| Specific rotation | In chloroform $[\alpha]_D^{25}$ + 160° |
| | In acetone $[\alpha]_D^{23}$ + 152.2° |
| UV absorption spectrum (in ethyl alcohol) | maximum = 240 ± 0.25 mμ<br>log E = 4.20 (aldosterone monohydrate) |
| Solubility in water | = $1.2 \times 10^{-4}$ moles/l |

### Sulphuric Acid Fluorescence

Aldosterone fluoresces (poorly) in sulphuric acid, due to the presence of a hydroxyl group at C-11 and an $\alpha\beta$-unsaturated ketone group in ring A, giving a peak at 288 mμ after 2 h (Simpson et al. 1954a, b). The addition of phosphoryl chloride considerably improves the fluorescence (Touchstone et al. 1959). Sulphuric acid fluorescence has been used as the basis of a chemical assay (Carr and Reddy, 1961; Bruinvels and Noordwijk, 1962).

### Sodium Hydroxide Fluorescence

Fluorescence in ultraviolet light at 560 mμ is produced by aldosterone in concentrated sodium hydroxide (Bush, 1952) and was used in the original isolation: