VASOPRESSIC FACTORS
AND
THE DEVELOPMENT OF STEROID THERAPY
IN
NEPHROSIS

BY

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PREFACE

Prior to 1951 nephrosis was a disease characterised by capriciousness of course and unpredictability of outcome. The prognosis of the nephrotic patient was thought to be very poor and the multiplicity of alleged remedies in use served but to underline the inefficacy of any one of these.

In 1951 the newly discovered technique of paper chromatography attracted attention to the "nephrosis peptide", and synchronously ACTH and cortisone became available for clinical trial. It was decided therefore to undertake a tripartite investigation, firstly into the nephrosis peptide, secondly into the natural history of nephrosis in the children seen at The Royal Hospital for Sick Children, Glasgow, and thirdly, into the modifications produced by steroid therapy in the pattern of disease.

The chromatographic investigation of the nephrosis peptide and the active peptide of posterior pituitary extracts led to the biological assay of a variety of vasopressic agents during the period 1951-56. This series of experiments are described in Part I of the thesis. The attempt to establish the natural history of nephrosis as seen at the hospital was begun at the same time and the tracing of patients took eight years to complete. The results of this investigation are given in Part II. Treatment with ACTH and cortisone began in a very tentative fashion in 1951. As our knowledge/

knowledge increased so also did the level and duration of dosage and the purity and potency of the available preparations. The newly acquired knowledge of the natural history of the disease enabled the effects and value of steroid therapy to be assessed accurately during the period 1951-1959. The series of therapeutic tests carried out have been described in chronological sequence in Part III. Treatment with prednisolone which was devised and tested here has been widely accepted.

Throughout this work Professor Stanley Graham has been a constant source of help and encouragement. Both he and Dr. J.H. Hutchison made clinical cases available and these children were nursed in the wards of The Royal Hospital for Sick Children, Glasgow.

I am indebted to Dr. H.E.C. Wilson for instruction in the partition chromatography and his laboratory carried out the routine biochemical investigations of the patients. Dr. J.D. Dekanski taught me the technique of pressor bioassay in the rat. With each of these colleagues I have published joint scientific communications. The almost incredible feat of Miss M. Walker who traced every single one of 164 cases of nephrosis occurring between 1929 and 1957 merits the highest praise. A number of these children were scattered over five continents and the complete follow-up of them involved the willing cooperation of many professional colleagues to whom I am indebted.

The contents of this thesis have been composed in their entirety/

entirety by myself, unaided. The clinical work, the bioassays, and the chromatographic investigations were done by myself and routine biochemistry was carried out by the hospital laboratory as acknowledged above. A list of relevant publications is given at the beginning of the bibliography.

PART I

EXPERIMENTAL INVESTIGATIONS

PEPTIDE STUDIES

In 1948 Dent, using two-dimensional paper chromatography, detected an unusual peptide in urine from patients with nephrosis. This finding was confirmed on numerous occasions during the period 1951-55 at the Royal Hospital for Sick Children where the "nephrosis" peptide was found in the urine of such patients. The quantity present varied considerably from patient to patient, and in the same patient from time to time. Similar peptide was found in the urine of patients with oedema due to other causes such as acute haemorrhagic nephritis, anaphylactoid purpura, polyarteritis nodosa, and pre-eclamptic toxaemia. It was noted in the urine of patients who had sustained trauma such as a burn, a scald, or a fracture, or who had undergone a surgical operation (Arneil and Wilson, 1953). Barlow (1952) reported the presence of excessive antidiuretic activity in the blood of oedematous patients with nephrosis and this finding suggested the possibility that this urinary peptide might be related in some way to antidiuretic activity. It was therefore decided to examine some commercial extracts of the posterior-pituitary gland to establish whether any peptide was present and if so to determine whether or not it possessed antidiuretic activity. Eight posterior pituitary preparations in every day use as antidiuretic and oxytocic agents were subjected to two-dimensional filter-paper chromatography. The technique described by Consden et al. (1944) was used firstly to analyse the amino-acid and peptide structure of the/

the extract, and secondly to separate the components for individual investigation. The nephrosis peptide (as described by Dent) had a high Rf. in phenol and a very low Rf. in butanol-acetic acid.

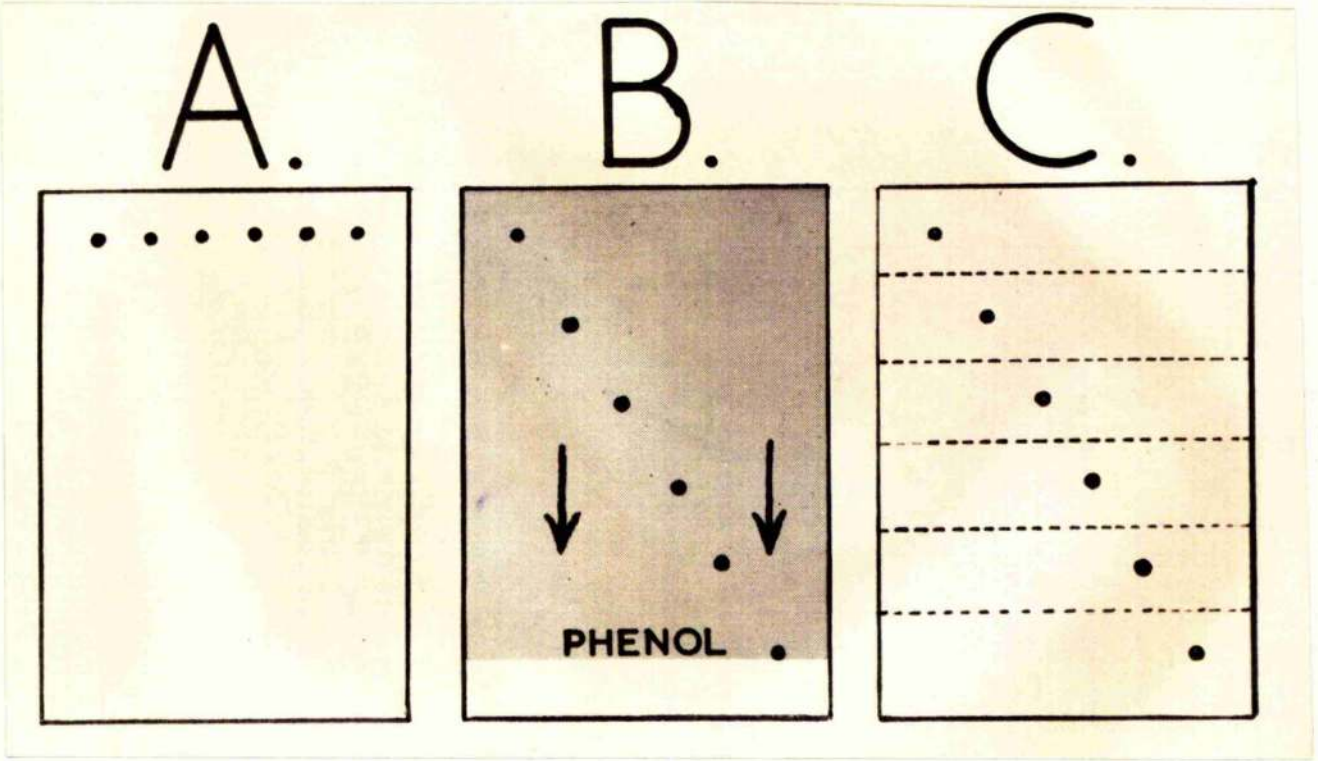
METHODS

0.5 ml. of the liquid pituitary extracts were pipetted onto a spot two inches inside the corner of a sheet of No. 1 Whitman's filter paper. The preparation was run with phenol ammonia as solvent, then turned 90 degrees and run with butanol and acetic acid. The resultant was developed with 'Ninhydrin' and the various fractions present could then be identified by their disposition on the filter paper.

In order to isolate the different components of the commercial extracts for separate testing the following method was devised. The contents of an ampoule of the extract were pipetted as a line of spots three inches from the edge of a sheet of filter paper. This preparation was then run in phenol ammonia for about forty hours, by which time the phenol edge had moved about fifteen inches from the line of application of the material. This fifteen-inch length of paper was next cut into six or seven strips at right angles to the line of flow of the phenol. The strips were eluted separately by capillary flow of distilled water so that each eluate contained the amino-acids or peptides according to their Rf. in phenol (Fig. I). The remaining preparation was a snuff and was first dissolved in 2 ml. of distilled water, centrifuged, and the supernatant/

FIGURE I

Diagrammatic Representation of a Simple Chromatographic Method of Separating Various Substances in Solution.



A = Solutions applied as a line of spots on filter paper prior to running

B = Phenol run in progress: substances travelling with phenol in proportion to solubility in the phenol ammonia

C = Paper dried and about to be cut in strips at right angles to flow of phenol

Note: For simplicity the various substances are shown separately: in practice each spot consists of a mixture of the substances.

supernatant fluid then applied to the paper in the same way as before.

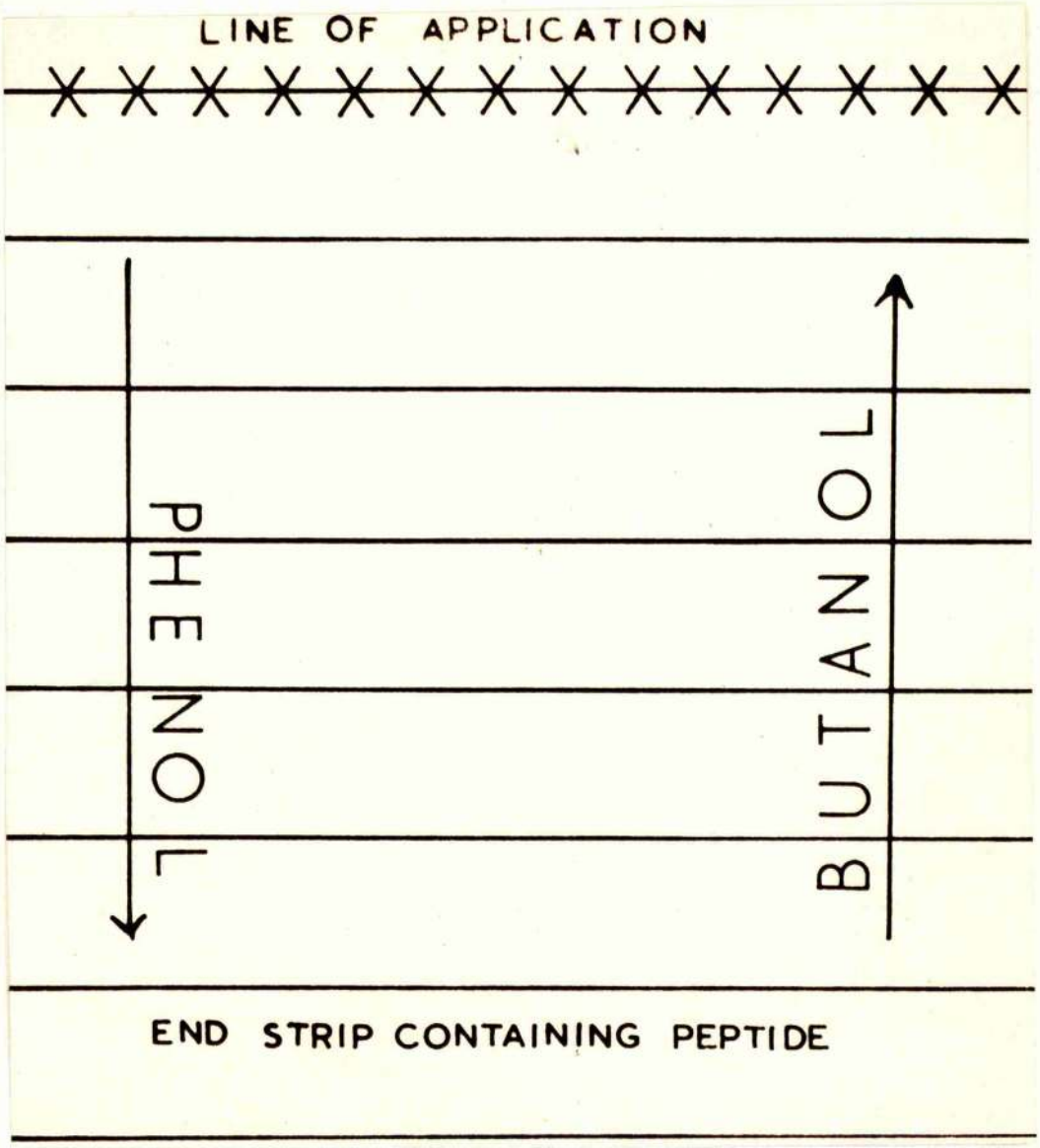
When it was desired to obtain a pure preparation of polypeptide from the end strip, the following technique was adopted. The contents of an ampoule were pipetted in a line of spots as before, run in phenol ammonia, and the paper was then reversed and run back in butanol acetic acid for twenty-four hours (Fig. II). By this means traces of amino-acids such as arginine and lysine, which would otherwise have lain close to the end strip of a simple phenol run were separated and any acetylated amino-acids which might have been present were removed. The nephrosis and pituitary peptides being insoluble in butanol remained static (Fig. II). The end strip was eluted and the eluate divided into two portions - the one being subjected untreated to partition chromatography, the other hydrolysed in 6N. hydrochloric acid for thirty-six hours, neutralised and then similarly investigated.

In order to obtain peptide from urine for analysis and assay the following method was employed. The urine was deproteinised by reising its temperature to 100°C, adjusting to pH 5.5 and filtering when cool. The filtrate was then treated with sodium tungstate and sulphuric acid (which was found to precipitate the peptide). The precipitate was centrifuged, washed in water, recentrifuged, and dissolved in ammonia. This concentrate was pipetted on to the paper in a line of spots as described above, run in the same solvents as were used for the nephrosis peptide and eluates prepared for assay. By this means the residue of 20 to 30 ml. of urine

could/

FIGURE II

Diagrammatic Representation of Method Used to Obtain Samples of Substances Highly Soluble in Phenol Ammonia but not in Butanol and Acetic Acid.



The end strip is removed and then eluted with distilled water.

could be applied to one sheet of paper. A solution containing the tungstate and sulphuric acid only was run under identical conditions, corresponding strips eluted, and the product tested as a control.

RESULTS

Posterior-Pituitary Extracts

A substance highly soluble in phenol but not in butanol was present on chromatographic analysis of each of the eight commercial preparations. This substance occupies the spot "L" as shown in Fig. III, which is a photograph of an actual bi-directional chromatograph. This substance has a high Rf. in phenol and a low Rf. in butanol acetic acid.

For the following reasons this unknown substance "L" is thought to be a polypeptide.

(1) The substance is not a protein, being soluble in phenol ammonia.

(2) The substance can be dialysed through a 'Cellophane' membrane and retain its form and activity.

(3) The substance when hydrolysed yields at least twelve amino-acids. These are arginine, leucine, isoleucine, valine, aspartic acid, glutamic acid, cystine, lysine, glycine, alanine and traces of threonine and serine. (Fig. VII).

The presence of such peptide in these extracts having been established/

established the substance was tested for antidiuretic activity. Strips of chromatograph paper were prepared as described, eluted by distilled water and each eluate reduced in volume to 1 ml. The antidiuretic activity of the eluate of each strip was assayed in rats by the method of Burn (1931). At the same time duplicate papers were developed with Ninhydrin to display the position of the various amino-acids and peptides. The time of half-diuresis (the time between the gavaging of 5 ml. of water per 100 g. of rat and the excretion of 50 per cent. of this fluid as urine) was arbitrarily accepted as the critical measurement. The results obtained on testing eight different extracts are shown in Table I.

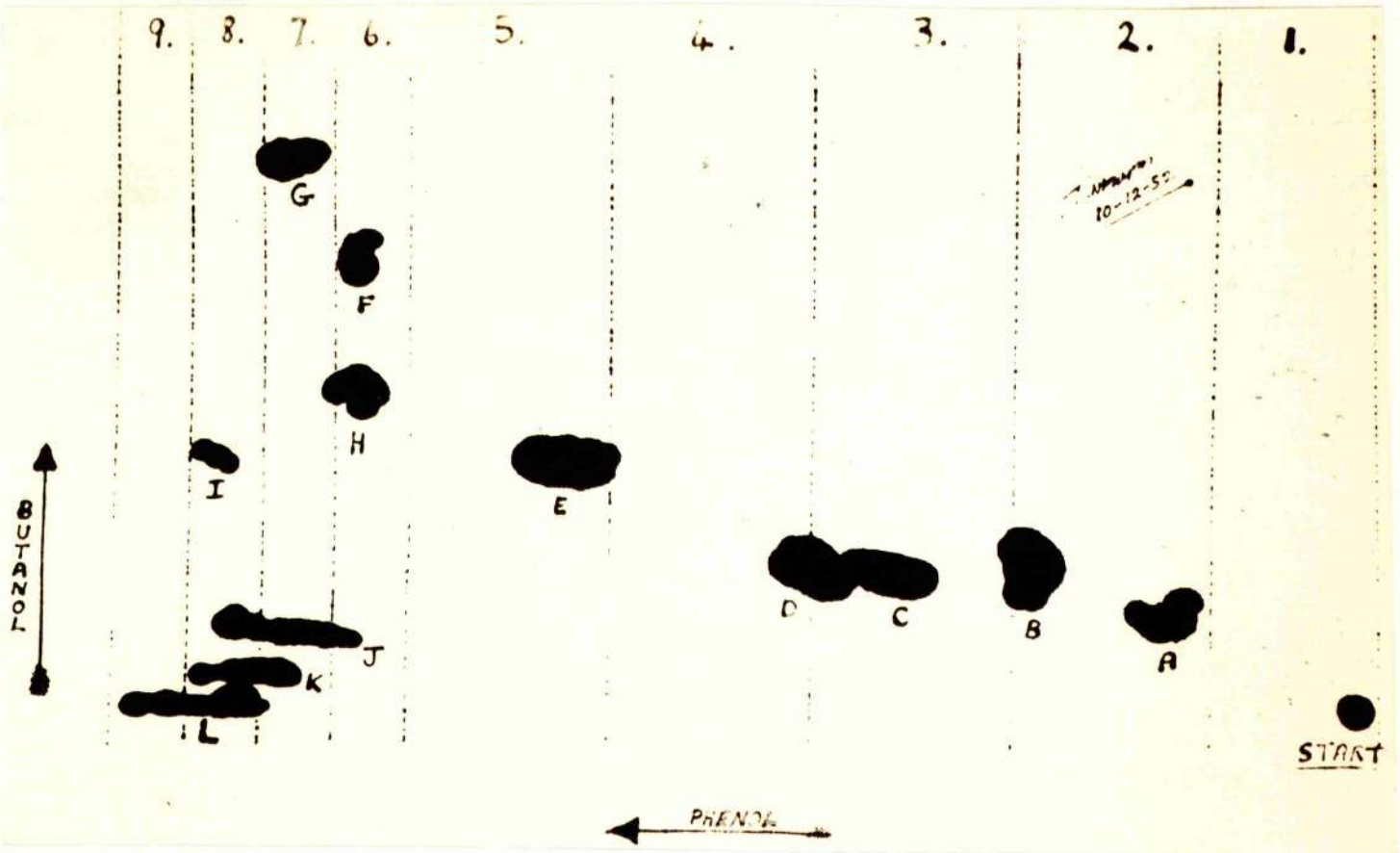
In each series the eluate of the end strips of paper (which had been shown on duplicate sheets to contain the peptide) possessed strong antidiuretic activity remarkably potent in view of the minute amount of the substance present (Table I). On two occasions the chromatograph paper was cut into strips from one end to the other and the eluate of each strip in turn was tested for antidiuretic activity. In each case antidiuretic activity was present in the end strips and in neither case was antidiuretic activity present in a strip which did not contain the peptide (Fig. III and IV).

At a later stage the more delicate method of rat bioassay (as described later in this thesis) was employed and the results of using this method to test the strip elutions is shown in Fig. V. When the potent eluate of such end strips, shown to contain the peptide, were tested following hydrolysis with 6N HCl, so that the

peptide/

FIGURE III

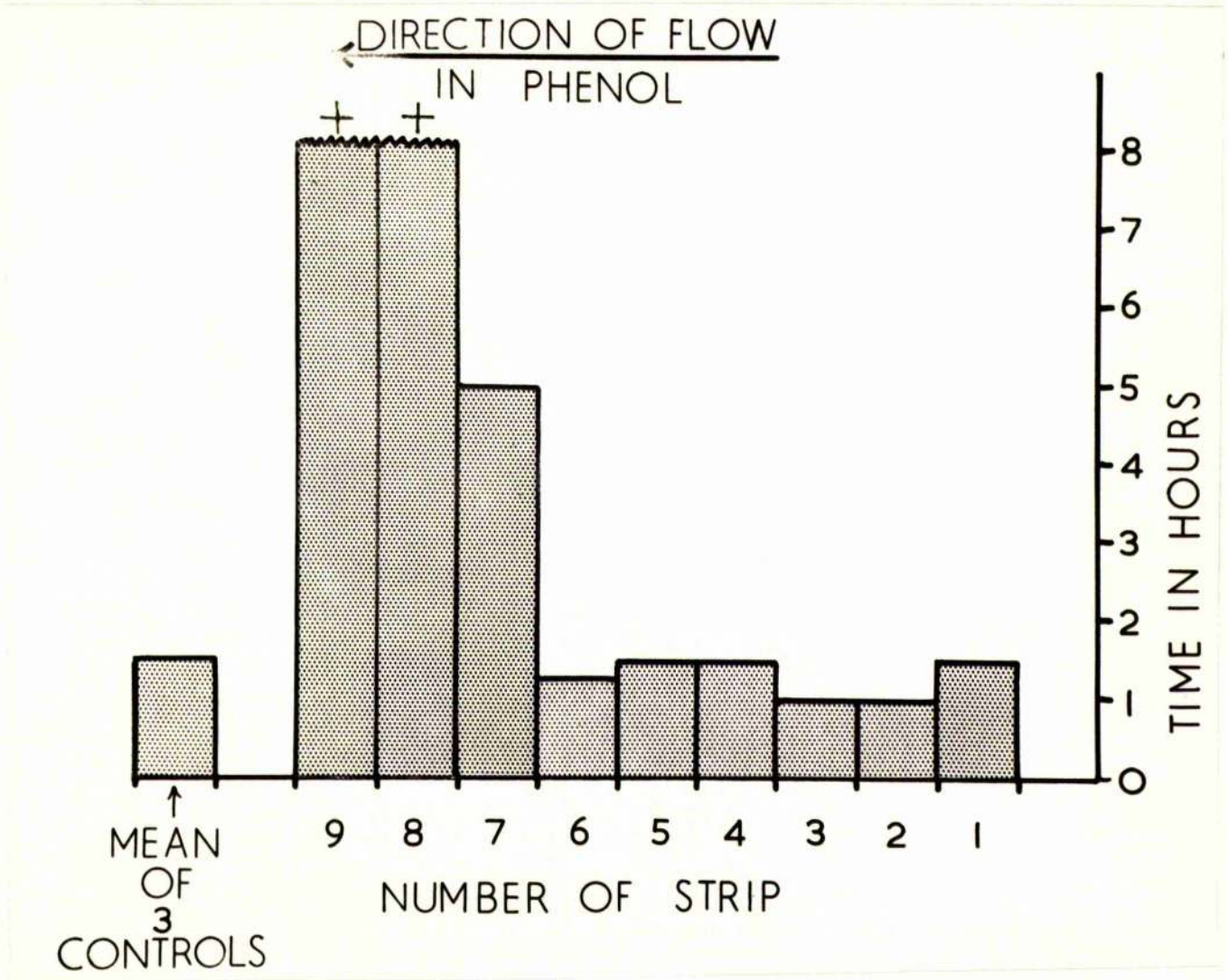
Photograph of Bi-directional Chromatogram of Posterior Pituitary Extract.



Eleven amino-acids (A - K) and one peptide "L" were present. The dotted lines coincide with the lines of cleavage in an exact duplicate sheet which was cut into strips, each separately eluted with distilled water and tested for antidiuretic activity. (Fig. IV).

FIGURE IV

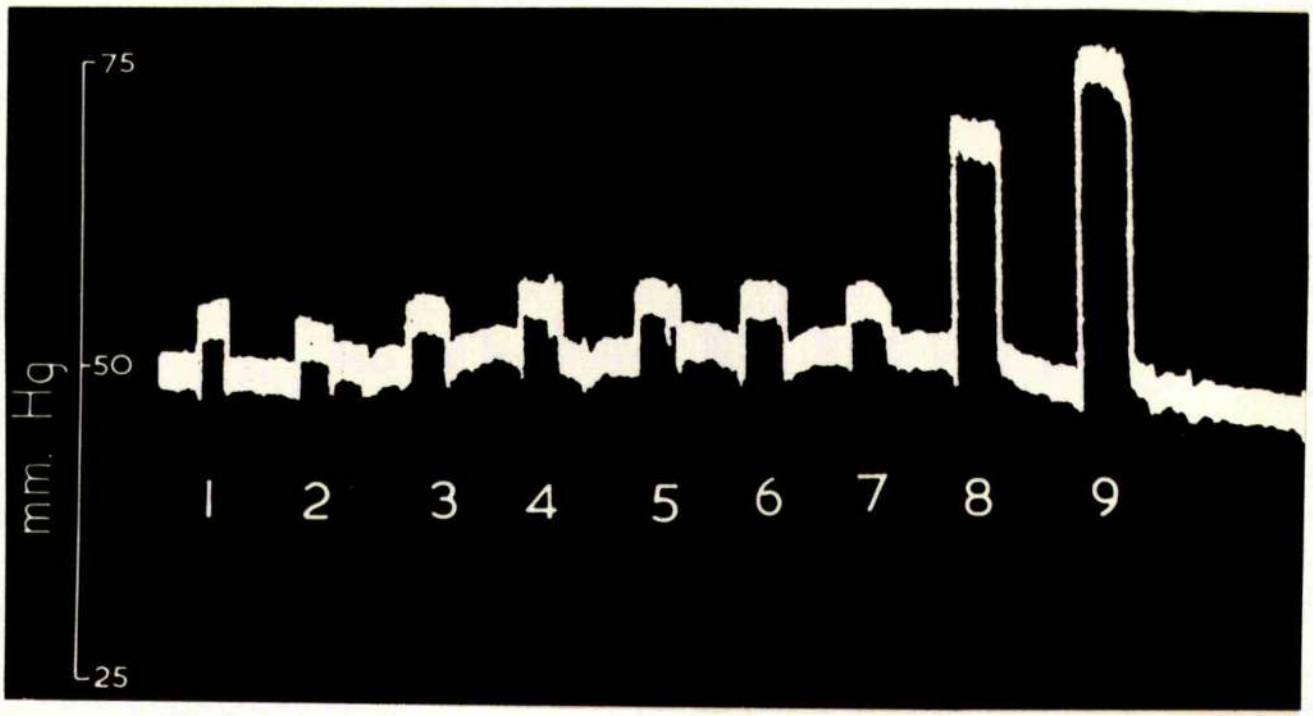
Antidiuretic Response of Rats to Eluates of Strips Prepared from Chromatogram of Posterior Pituitary Extract.



The antidiuretic response is confined to the strips in which the peptide "L" (Fig. III) was present. The time refers to time of half-diuresis in rats (see context).

FIGURE V

Vasopressic Response of Rat to Intravenous Injection of Eluate from Serial Strips of a Chromatographic Run Similar to that shown in Fig. III.



This is a continuous tracing of the blood pressure of a dibenamised rat. The peaks are produced by the end strips of the phenol run.

TABLE I

Antidiuretic Activity of Peptide Extracted from Commercial Posterior-Pituitary Preparations by Chromatography Tested in Rats by Estimating Time of 50 per cent. Diuresis. (After Burn, 1931).

Preparation	Peptide in strip	Time of 50% diuresis (hr.) 1	2	Control	Control	Anti-diuretic activity
A	Yes	8+	8+	1.1/2	1.3/4	+
B	Yes	8+	8+	1.1/2	1.1/2	+
C	Yes	4.1/2	6	1.1/4	1.1/2	+
D	Yes	6.1/2	3.1/4	1.1/2	1.1/4	+
E	Yes	3+	5+	1.3/4	1	+
F	Yes	7+	3.1/4	1.1/2	1.1/4	+
G	Yes	4	7+	1.3/4	1.1/2	+
Snuff	Yes	3	6+	1.1/2	1.1/4	+

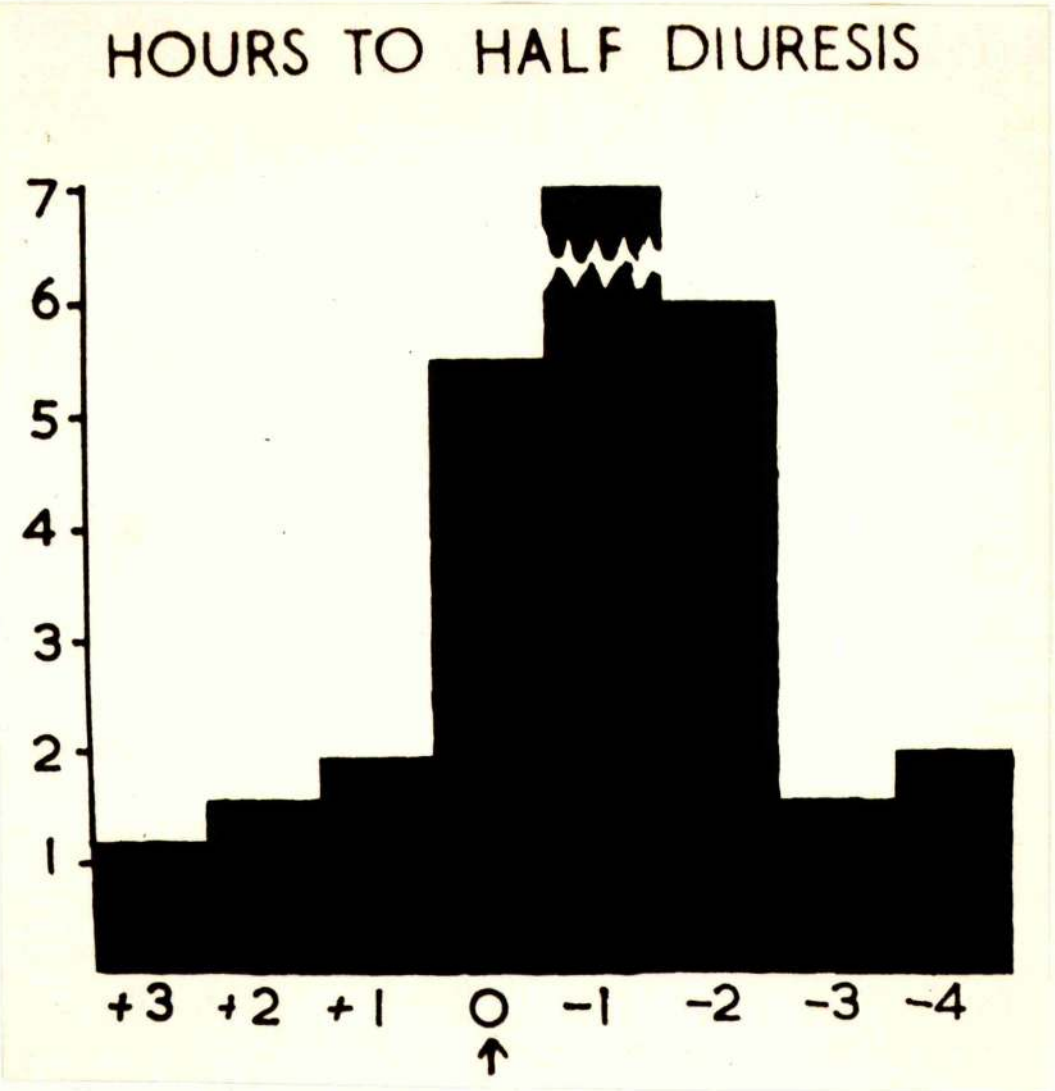
peptide was no longer present, the antidiuretic activity had disappeared.

Electrophoresis of the pituitary extracts (using the method of Cremer and Tiselius) was then carried out. The peptide was shown by Ninhydrin staining to have moved towards the negative pole. Strips of filter paper were again cut at right angles to the flow and each strip was separately eluted and the eluates tested for anti-diuretic activity. The results of one such test are shown in Fig. VI.

The/

FIGURE VI

Antidiuretic Activity of Eluates from Serial Strips from Filter Paper on which Posterior-Pituitary Extract has been Subjected to Electrophoresis.



The antidiuretic activity as shown by the time of half-diuresis in rats is confined to the area wherein the peptide lay in a duplicate sheet stained with Ninhydrin.

The antidiuretic activity was confined to the area wherein the peptide lay, as shown on duplicate sheets.

It therefore seems that this peptide contains the antidiuretic fraction of these preparations from the posterior lobe of the pituitary gland. No antidiuretic activity was detected in any other component of the extracts, and if the peptide was hydrolysed the antidiuretic activity was lost. The presence of at least twelve amino-acids, some in greater concentration than others, makes it likely that the molecular weight of the substance lies between 1200 and 2000. The finding of Turner et al. (1951) that the very pure vasopressin which they analysed contained only eight amino-acids suggests either that the peptide described herein is a less advanced stage in the breakdown of the macro-molecule, or that some other substance, such as the oxytocic factor, has run identically in all these tests. A high concentration of similar peptide was found in human pituitary gland, and small traces have been found in other organs.

Urinary Peptide

"Nephrotic peptide" obtained from the urine of patients with acute haemorrhagic nephritis, nephrosis and pre-eclamptic toxaemia was then subjected to a similar investigation. Certain problems arose before successful chromatographic results were obtained, owing to the necessity of removing contaminants, such as albumin. On chromatography, the "nephrotic peptide" behaved in a similar fashion

to/

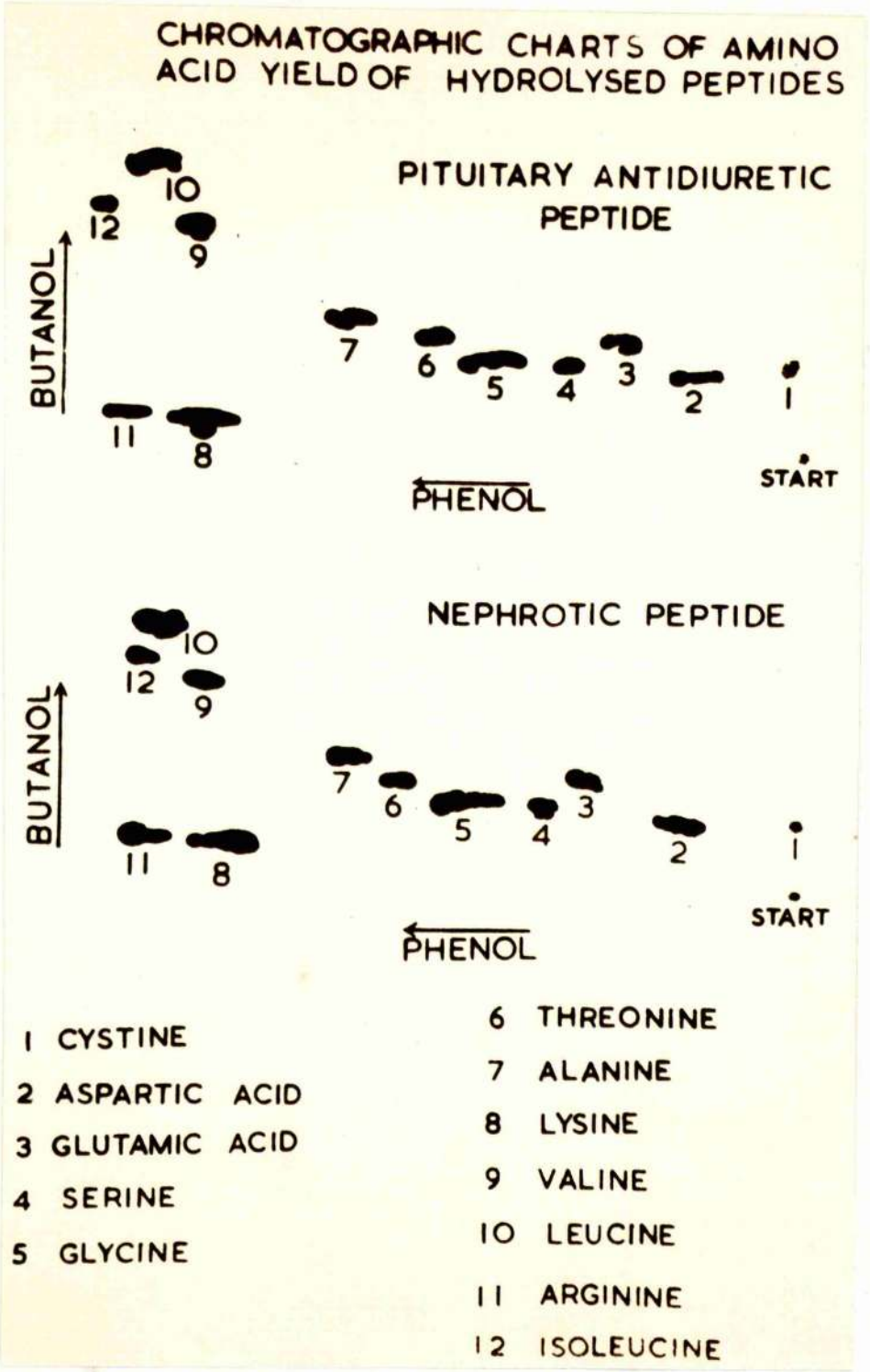
to the posterior-pituitary peptide. Hydrolysis showed that it contained the same twelve amino-acids and the proportions appear comparable. The similarity of the pattern formed by the amino-acids of the two peptides is shown in Fig. VII. When the nephrotic peptide and the posterior-pituitary peptide are subjected to electrophoresis they both move towards the negative pole (Fig. VIII) whereas ACTH (a similar peptide) does not.

"Nephrotic peptide" from the urine of seven patients was then injected subcutaneously into rats. This was done on twenty-two occasions, and in only three instances did a well-marked antidiuretic response result. It has been stated by Pickford (1952) that the method used was crude, testing only to 20 mU of antidiuretic activity, whereas the method of Jeffers et al. (1942) will detect 0.02 mU of antidiuretic activity. Though further observations with a more sensitive method are essential, it is clear that this urinary peptide has much less antidiuretic activity than the pituitary peptide. On eight of the twenty-two occasions on which "nephrotic peptide" was injected subcutaneously into rats, convulsions ensued shortly thereafter. These convulsions occurred within five minutes of the injection, lasted up to two hours, and appeared to leave no permanent damage (although large doses of postoperative urinary peptide killed some of the animals). This convulsant effect is much greater if the peptide is given intraperitoneally and may be a result of traces of phenol combining with peptide therefore persisting.

The following test was made on two volunteers who were /

FIGURE VII

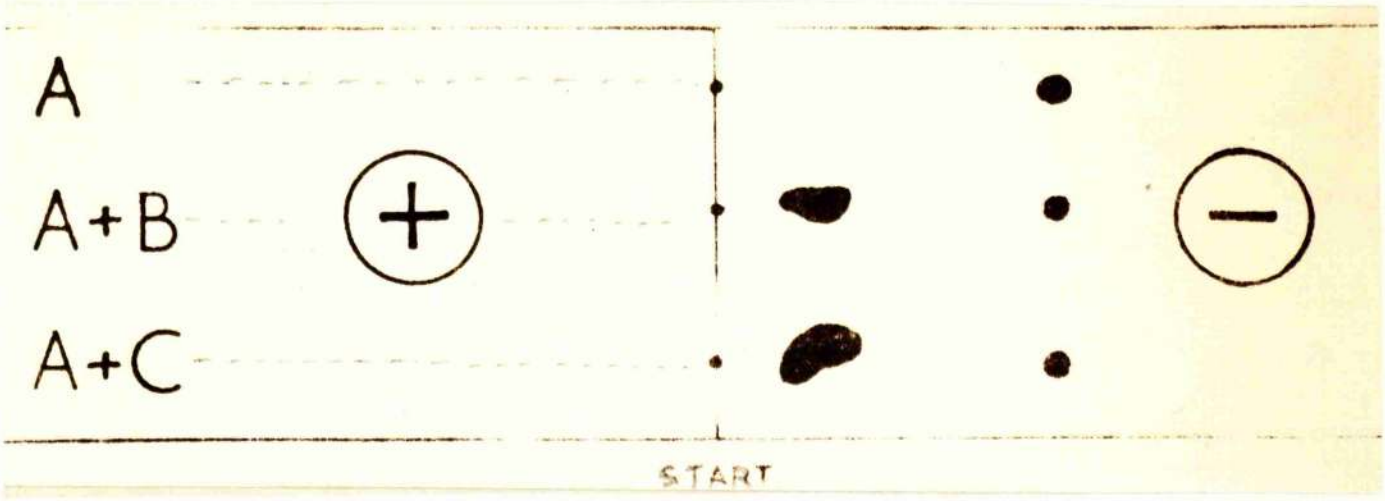
Comparison of Amino-Acid Yield Following Hydrolysis of Pituitary Peptide and Urinary Peptide.



The two peptides are seen to contain the same amino-acids.

FIGURE VIII

Comparison of Electrophoretic Movement of Pituitary Peptide and Nephrosis Peptide.



A = control, arginine spot only

B = pituitary peptide + arginine

C = nephrosis peptide + arginine

The arginine is used to control the relative distance run from each spot.

were non-smokers. Each drank a litre of warm water and when brisk diuresis had started, smoked a cigarette, inhaling deeply and producing a well-marked antidiuretic response. No peptide was detected directly by chromatography of the urine passed by either person before smoking, and no antidiuretic or convulsant effect was demonstrated on injection of eluates into rats. The urine passed after the "smoking antidiuresis" in subject G.A. possessed antidiuretic activity; and, although the "nephrotic peptide" was not visible when 0.5 ml. of urine was run, the presence of this peptide was clearly shown by the amino-acid yield of hydrolysis of the eluted end strip of a double-run chromatogram. In subject C.G. the "nephrotic peptide" was easily seen, antidiuretic activity was present, and the end strip of a sulphuric acid-tungstate precipitate of this urine caused convulsions in rats. It is presumed that the inhaled nicotine released an excess of antidiuretic hormone from the posterior lobe of the pituitary gland, antidiuresis ensued and thereafter the presence of urinary peptide was detected. The finding that the eluate of the strip containing this urinary peptide from a normal person caused convulsions in rats strongly suggests that this is not an unaltered pituitary peptide.

DISCUSSION

The following possible explanation of the results so far obtained is tentatively advanced. Several of the hormones of the pituitary/

pituitary gland - e.g. ---- oxytocin and vasopressin - are peptides with a similar though not identical amino-acid content. Small quantities of such peptide can be found in the urine of normal people, and the quantity present may be increased by inducing anti-diuretic activity with nicotine. In a group of diseases characterised by oliguria and oedema and renal damage (nephrosis, acute haemorrhagic nephritis, and pre-eclamptic toxaemia) a considerable quantity of similar peptide is found in the urine. Some of these urinary peptides closely resemble the peptide present in the posterior lobe of the pituitary, behaving similarly on chromatography, hydrolysis and electrophoresis. These properties are shared by a variety of similar peptides. Where haematuria exists the peptide split off haemoglobin by phenol must be differentiated by its structural variation from the nephrosis peptide since its chromatographic behaviour is identical. They differ in their antidiuretic activity in rats. It seems likely that at least some part of the urinary peptide is the altered form in which the antidiuretic peptide of the posterior lobe of the pituitary gland is excreted. In the presence of disease complications such as the existence of similar peptides derived from the pituitary gland, haemoglobin or other sources, failure of the liver to dispose of peptides, and failure of the nephron to maintain peptides in the plasma must inevitably arise. Byrom (1938) has shown that oestrin will increase tenfold the sensitivity of the rat's kidney to damage by vasopressin. It seems possible/

possible that an abnormal balance of hormones, together with an excess of pituitary antidiuretic peptide, may be concerned in some way with the production and course of the disease characterised by the presence of oedema and of an excess of such urinary peptides. Pickford (1952) takes the view that the adrenal overactivity precedes that of the pituitary, in a number of conditions in which an excess of antidiuretic substances have been found circulating. Whether this order of events be correct or not, the occurrence of this peptide (which we believe to be derived from the posterior pituitary hormone) in the urine of these patients seems to confirm her theory of excessive antidiuresis. It does not appear impossible that the influence of excessive antidiuretic hormone may contribute to the onset of oedema in such diseases as nephrosis. The excretion of increased quantities of this urinary peptide following trauma or surgical operation suggests the possibility that an excessive output of pituitary hormone may occur at this time and such water retention has in fact been demonstrated by Le Quesne and Lewis (1953), Le Quesne (1954) and Eisen and Lewis (1954). Furthermore, it has been shown that various species react differently to heterologous antidiuretic hormone. Man is, weight for weight, twenty times more sensitive than rat to the antidiuretic hormone of the ox (Burn, 1931). It may well be that human antidiuretic hormone is a less efficient antidiuretic in rats. With the possibility in mind that excessive protein katabolism or other factors contribute it is suggested/

suggested that such substances in urine be termed a "peptide group" until more information is available. Since our observations the similarity of the pituitary and urinary peptides has been observed by workers in Italy using almost identical methods ((Valeri, Zacco and Perrini (1953)). These peptides raise many interesting problems. Since it seemed possible that their presence in disease was due to an excessive excretion of a physiological end-product, it was considered important that quantitative methods of estimating the daily output in urine should be devised. Many fruitless attempts to perfect such a method were made and the problems attending the evolution of such a method proved immense. It was decided therefore to try to devise a more delicate method of estimating the vasopressic activity of plasma, in the hope that differing levels in health and disease might yield helpful information.

CONCLUSIONS

By paper chromatography a peptide containing at least twelve amino-acids has been extracted from eight different commercial posterior-pituitary preparations. This peptide has strong anti-diuretic activity and when the solutions are divided into their component parts it is the only part of these extracts possessing this property. It probably is or contains the antidiuretic factor.

The "nephrotic peptide" is present in the urine of oedematous patients/

patients with such diseases as nephrosis, acute haemorrhagic nephritis and pre-eclamptic toxaemia. On chromatography, electrophoresis and hydrolysis followed by chromatography this peptide behaves like the posterior-pituitary peptide; but on injection into rats it seldom inhibits diuresis and may cause convulsions, probably due to phenol.

"Nephrotic peptide" was found in the urine of two normal people after diuresis had been induced by nicotine and on injection into rats it caused convulsions.

PRESSOR ACTIVITY OF PLASMA FROM HYPERTENSIVE NEPHRITICS

A tentative suggestion that pituitary vasopressin might contribute to the maintenance of oedema and be excreted as a peptide in the urine having been mooted, it was decided to establish whether an excess of vasopressic activity existed in the plasma of patients with such peptiduria. Since the greatest amount of the "nephrosis" peptide seemed to be present in urine from patients in the hypertensive stage of acute glomerulonephritis plasma from eight such cases was tested. In each instance the blood pressure exceeded 125/95 mm. of mercury and "nephrosis" peptide was present in the urine of each patient. In no case was a previous history of renal disease obtained. Blood was removed from time to time during the course of the illness and after the patient had been asymptomatic for some time.

METHODS

Five millilitres of systemic venous blood were removed from the antecubital vein of the patient and to this blood 500 units of heparin were added. The specimen was at once centrifuged for 10 minutes at 1500 revs./minute and the supernatant plasma removed and chilled to 4°C. It was then packaged and despatched overnight to the laboratory.* Control samples of plasma were obtained from healthy children/

*The estimations of vasopressic activity of plasma from these eight cases was made by Dekanski at Organon Laboratory on my request.

children of comparable age in a similar fashion. The pressor activity of the plasma was estimated at first by the method of bio-assay for vasopressin described by Dekanski (1952) and later by a modified technique for angiotonin. Samples of plasma to be tested by the latter method were treated with sodium thioglycollate to 0.01 molar concentration for thirty minutes before assay began. The pressor activity was at first measured against International Posterior Pituitary Lobe Standard and later against Solution Angiotonin. Throughout this book the term "cat unit" is used to refer to the "Eli Lilly" cat unit of angiotonin defined as follows:-

"The standard preparation is a vacuum dried powder thirty microgrammes of which are designated as one unit. It corresponds approximately to that amount which produces a rise of roughly 30 - 50 mm. in the blood pressure of the pithed cat. Great difficulty is experienced in standardising because from animal to animal and moment to moment angiotonin does not give comparable pressor responses to any other known pressor agent." This unit is one tenth of that proposed by Braun-Menéndez and other South American workers since 4 Lilly cat units can be derived from one millilitre of plasma whereas 0.4 (Braun-Menéndez) units are produced by a like amount. The Braun-Menéndez unit of angiotonin is that which produces a rise in carotid blood pressure of 20 - 30 mm. of mercury in an average chloralosed dog weighing 10 kg. (O.M. Helmer, personal communication 11.9.53.)

A qualitative expression of the various types of response obtained when/

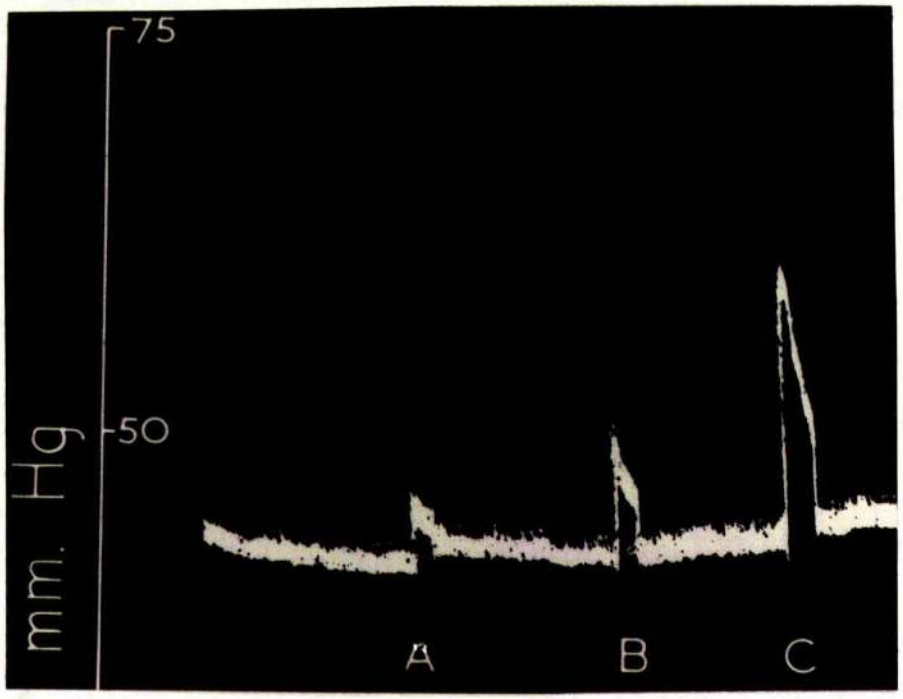
when plasma is injected intravenously into the rat is given in Figure IX. The response to 0.4 ml. of plasma from a control subject (A) was little greater than the response resulting from a like quantity of normal saline: the response from 0.4 ml. plasma of a patient with acute nephritis and hypertension (B) is greater and markedly augmented when the plasma has previously been retained for twelve hours at 37°C.

This simple visual comparison gave way to a four point method of assay in an endeavour to obtain absolute values capable of valid comparison. Injections were given in a series of groups of four, each group consisting of a high and a low dose of the known solution (vasopressin or angiotonin) and two differing volumes of the plasma. The large dose was generally double the small dose and the order of injection was varied from group to group. In Figure X quantitative comparison of the pressor activity of plasma from a hypertensive patient and Solution Angiotonin is produced. By measuring such recordings an approximation of the absolute quantity of pressor activity could be arrived at by calculation (Arneil and Dekanski, 1954).

The vasopressic activity of the plasma from these eight patients ranged from 51 - 118 cat units per 100 millilitres of plasma. These values are shown in Table II which also includes results obtained from plasma derived from these same eight subjects when restored to health and normotension. A number of samples from normal children were similarly assayed and these results are also shown.

FIGURE IX

Pressor Response to Various Injections of Plasma.

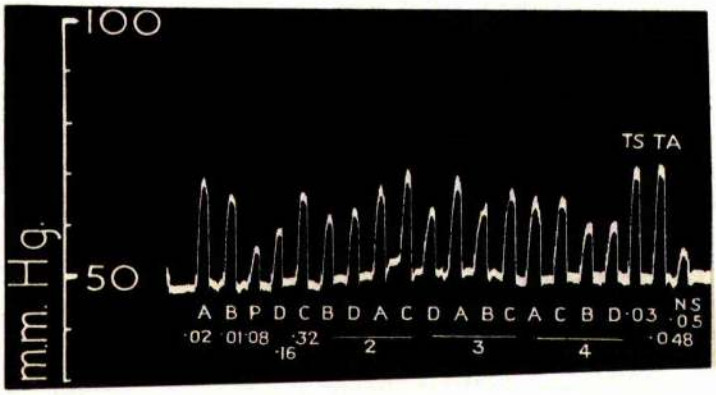


A = 0.4 ml. "control" plasma
B = 0.4 ml. hypertensive plasma
C = as B + 12 hours at 37°C

The smoked drum is not moving continuously.

FIGURE X

Quantitative Estimation of Pressor Activity in Plasma.



A = 0.2 c.u. angiotonin
B = 0.1 c.u. angiotonin
C = 0.32 ml. plasma
D = 0.16 ml. plasma

NS = 0.5 ml. normal saline
P = 0.8 ml. normal saline
TA = vasopressically active plasma + thioglycollate
TS = angiotonin + thioglycollate

TABLE II

Vasopressic Activity of Plasma Samples Expressed as Cat Units (Lilly) per 100 ml.

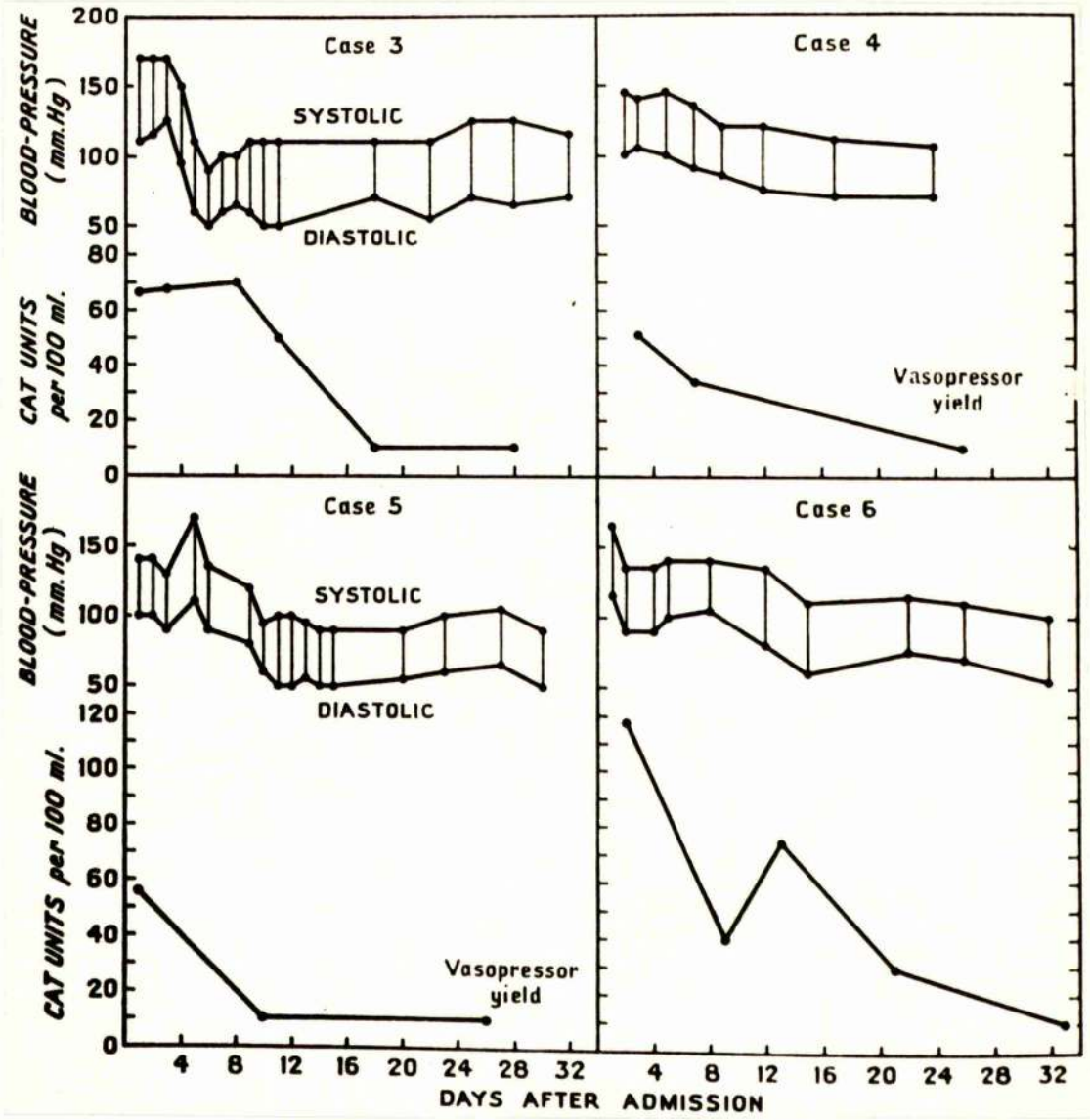
Acute Nephritis Group			Healthy Children	
Case	During Hypertension	After Recovery	No.	
1	55	<10	1	<10
2	61	<10	2	<10
3	67	<10	3	<10
4	51	<10	4	<10
5	56	<10	5	<10
6	118	<10	6	<10
7	62	<10	7	<10
8	57	<10	8	<10

The vasopressic activity in pathological plasma seemed to be greatest during the acute stage of the illness when hypertension, oliguria and oedema were most marked. This synchronism in four patients is illustrated in Figure XI: such parallelism may be coincidental and reflect a common relationship to the onset and course of the disease rather than a direct relationship between blood pressure and plasma vasopressic activity.

The absence of detectable pressor activity in plasma removed from healthy children is recorded in Figure XII. In a few instances unexplained vasopressic activity was noted in plasma from such children. For this reason the absence of vasopressic activity in plasma from formerly hypertensive patients was established in each case in an endeavour to preclude misleading results (Table II).

FIGURE XI

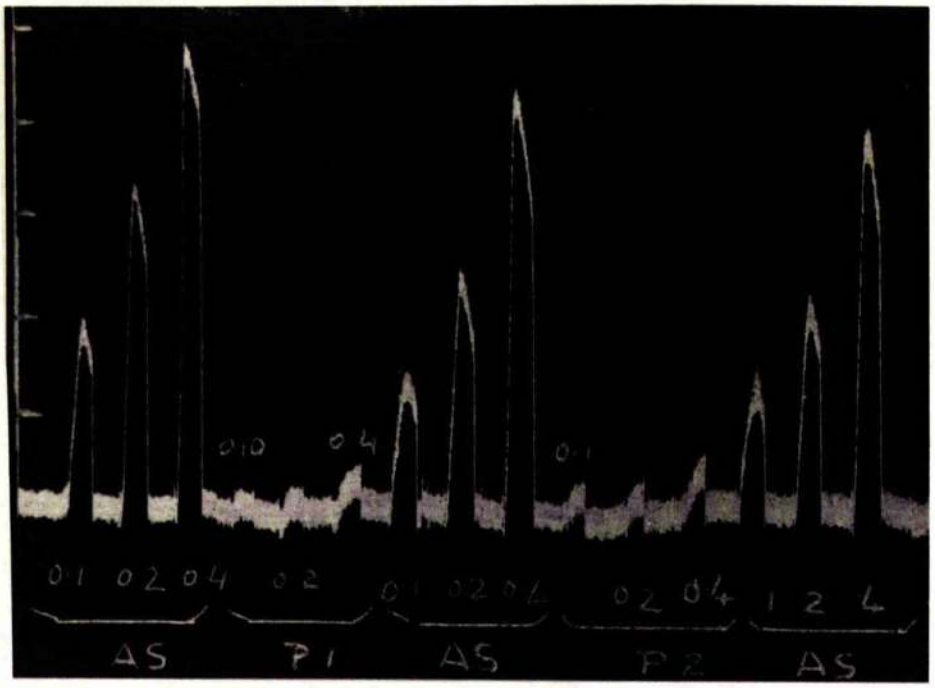
Relation of Systemic Blood Pressure and Plasma Pressor Activity in Four Cases of Renal Hypertension.



Note that the fall in blood pressure approximately parallels the decrease in plasma vasopressic activity, possibly due to a common relationship to the disease process.

FIGURE XII

Pressor Responses to Samples of Normal Plasma.



AS = Angiotonin 2 c.u./ml.

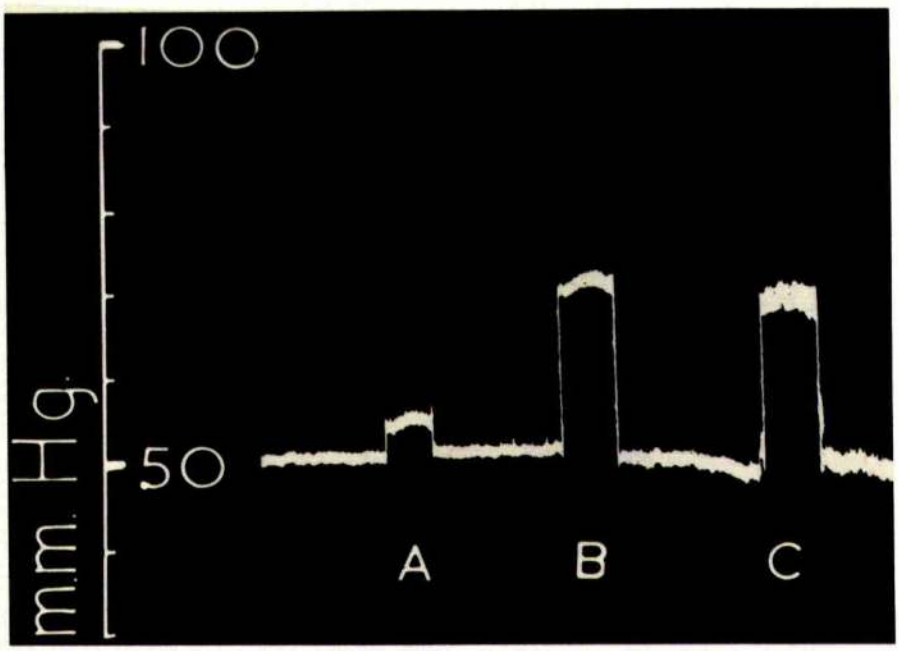
P_1 = Plasma 1

P_2 = Plasma 2

All figures refer to ml.

FIGURE XIII

Pressor Responses to Vasopressin



A = 0.4 ml. normal saline

B = 8 milli-units vasopressin

The smoked drum is not moving continuously.

There seemed little doubt that the systemic venous blood from these patients in the hypertensive phase of acute nephritis possessed considerable vasopressic activity. This pressor activity could not have been due to adrenaline, noradrenaline, iso-amylamine, piperidine or nicotine since the actions of these substances are blocked by dibenamine treatment of the rat preparation (Dekanski, 1951). The rise in blood pressure produced by vasopressin is more sustained and presents a quite different pattern (c.f. Fig. IX and Fig. XIII). Furthermore, if the plasma was treated to 0.01 molar with sodium thioglycollate for thirty minutes prior to assay no alteration occurred in the pressor activity (Arneil and Dekanski, 1954). The pressor effect was therefore not due to pituitary vasopressin which would be rendered inactive by this treatment.

Other vasopressic factors which occur in fresh plasma are the vasotonins, of which the most important is serotonin (5-hydroxy-tryptamine) (Rapport et al. 1948; Rapport, 1949). This substance exerts a depressor action on the blood pressure of the dibenamised rat (Dekanski, 1954) and would, consequently, tend to mask vasopressic activity rather than produce it. The possibility that the plasma pressor activity might be due to hypertensin* was then considered. Solution Angiotonin was obtained (through the courtesy of Messrs. Eli Lilly & Co., who flew supplies in from the United States when none was available in this country) and the pressor effects of this compared with/

*Throughout this text "hypertensin" is used to refer to the end product of the action of renin on hypertensinogen in vivo or in vitro and "angiotonin" to refer to Solution Angiotonin (Lilly).

with hypertensive plasma. Similarities were observed and solutions were noted to produce a rapid but evanescent rise in blood pressure: to be free of tachyphylaxis and to resist treatment with sodium thioglycollate.

At this point the mensurations made by Dekanski were repeated by me at The Royal Hospital for Sick Children, Glasgow. Divergent results were at once apparent; the plasma from the same eight patients (taken off during the hypertensive phase and retained at 4°C meantime) was not found to have the same excess of pressor activity which Dekanski had noted. It seemed that either the plasma had lost its vasopressic power or had failed to gain it. One possible explanation was that the activity had been augmented in vitro during transference through the post to the outside laboratory. A specimen of such plasma was therefore divided in two parts; one was retained at 4°C and the other at 37°C for twelve hours. The results of this test are shown in Figure IX and show the acquisition of hypertensive potency.

At this stage of investigation the possibility that plasma from patients with renal hypertension might contain an excess of renin which would act in vitro on plasma hypertensinogen under suitable thermal conditions and thereby produce an excess of hypertensin began to receive serious attention. In this disease the levels of hypertensinase and of hypertensinogen in the plasma have been shown to be within normal limits (Haynes and Dexter, 1945a & b). It was appreciated that other similar pressor systems might exist and it was decided to investigate fully the properties of plasma from normal subjects in this respect. Consequently/

Consequently the experiments described hereafter were undertaken. In order to appreciate the points involved a knowledge of the renin/hypertensinogen pressor system is essential and an outline is given below.

Since 1898 when Tigerstedt and Bergman reported pressor activity in saline extracts from kidney substance the possibility that a humoral factor might cause hypertension in human patients has attracted attention. Attempts to elucidate the point were ineffectual until 1934 when Goldblatt and his co-workers clearly demonstrated that continued renal ischaemia was followed by hypertension in dogs. The simple and reliable techniques which they devised gave a new impetus to research work on this subject in the experimental animal. The idea of producing ischaemia by restricting the renal afferent blood flow is not new and Katzenstein is reported as having noted slight elevation of the blood pressure of dogs following this procedure as long ago as 1905. Bridgman and Herose (1918) failed to confirm this finding following acute unilateral renal ischaemia but drew attention to the important results which might ensue when prolonged renal ischaemia was experimentally produced.

Goldblatt's technique had been applied to other mammals such as monkeys, rabbits and rats (Goldblatt, 1937; Pickering and Prinzmetal, 1938a; Kempf and Page, 1942). It therefore seemed highly probable that some such system existed in man and the search for evidence of a renal-pressor mechanism was continued.

In the experimental animal it was shown that this renal-pressor system/

system was humoral in type and exhaustive investigations were carried out. The mechanism appears to be as follows, (although lack of completely pure test substances has delayed final proof). Renin is a protein-like substance present in normal kidney, the factor which Tigerstedt and Bergman had postulated in 1898. Renin may be prepared as a powder from animal and human kidney extracts and different methods have been employed. The most useful methods seem to be those described by Dexter, Haynes and Bridges (1945) and Haas, Lamfrom and Goldblatt (1953a). Renin is thought to act on plasma hypertensinogen to produce the active peptide, hypertensin, which is rapidly destroyed, the rate of destruction depending on the amount of hypertensinase present in the plasma. The level of hypertensinase varies greatly in different species and is low in the plasma of man and present in considerable quantities in his kidneys. Muñoz et al. (1939) are said to have been first to suggest the enzymatic nature of renin and this suggestion has been amply confirmed (Haas, Lamfrom and Goldblatt, 1953b). The level of renin in plasma may be measured directly by intravenous injection (Pickering and Prinzmetal, 1938a) or by a variety of indirect methods (Leloir et al. 1940a; Page, 1940a; Dexter, Haynes and Bridges, 1945). Renin from different species of animals varies in specificity and several different fractions of renin have been shown to exist (Haas, Lamfrom and Goldblatt, 1953c). The blood pressure response to renin is slow in onset, is prolonged and tachyphylaxis occurs (Pickering, Prinzmetal, 1938; Blacket/

Blacket et al. 1950). Hypertensinogen is normally present in the plasma in considerable quantities and is readily available for the reaction (Plentl and Page, 1943a). The source of plasma hypertensinogen is the liver (Page et al. 1941) and the level circulating seems to regulate production. It is found as a component of the alpha-2-globulin fraction. Various methods of assay are available but that of Haynes and Dexter (1943) is widely used.

Hypertensin (Angiotonin) the resultant of the interaction of renin and hypertensinogen consists of at least two polypeptides with vasopressic activity (Skeggs, 1954). It is difficult to purify and can only be identified by bio-assay (Page and Helmer, 1940; Page, 1942; Houssay and Taquini, 1938a and b; Braun-Menéndez et al. 1940; and Dexter et al. 1945). The unit of measurement varies with different workers but there is general agreement that tachyphylaxis does not occur when it is injected to the experimental animal. The unit which has been adopted in this work is the cat unit (Eli Lilly) (see above).

It is thought that hypertensin injected intravenously into man produces peripheral vasoconstriction and reduction in the cardiac output (Bradley and Parker, 1941; Wilkins and Duncan, 1941). Furthermore constriction of the efferent arteriole occurs as judged by the rise in glomerular filtration rate and fall in renal blood flow (Corcoran et al. 1940). The polypeptide contains at least five amino acids (glycine, alanine, leucine, aspartic acid and glutamic acid) and five others may be present (histidine, arginine, lysine/

lysine, tyrosine and threonine). (O.M. Helmer, personal communication 15.10.53.)

Attempts to demonstrate an excess of hypertensin in systemic plasma from a human patient with hypertension have not been convincing and this, the final proof, is yet to be obtained. Nevertheless indirect evidence accumulates: partial occlusion of the renal pedicle has been attempted (Quimby et al. 1945) and a clinical counterpart producing hypertension in nephroptosis described by McCann and Romansky (1940b). The most convincing finding however has been the beneficial results obtained following nephrectomy in patients with unilateral renal disease and hypertension (Langley and Platt, 1947, and Pickering and Heptinstall, 1953).

It is clear that no method of estimation of hypertensin, using animals such as the cat (Dexter et al. 1945) is likely to be sufficiently sensitive to detect the small rise per unit volume of plasma which is likely to be present in a human patient with hypertension. Thus an increase of one cat unit per ml. of plasma would represent a rise of 5,000 cat units in the total plasma volume of a human. It seems most improbable that a rise to such levels would ever occur.

For this reason it seemed prudent to seek a more sensitive method of measuring hypertensin. The sensitive method of bio-assay for pituitary vasopressin which had been described by Dekanski (1951) is well established and it seemed to me that a similar method could be/

be used to measure Solution Angiotonin. At this point Dekanski, at my request, began to try his method on some Angiotonin (Dekanski, 1954) supplied by me and the investigations now described were carried out synchronously by myself at the Royal Hospital for Sick Children.

METHODS USED IN THE ASSAY OF ANGIOTONIN (R.H.S.C.)

Materials Employed

Rats: Male albino rats of the Glaxo strain varying in weight from 240 - 320 g. were used exclusively.

N.N.-Dibenzyl-β-Chloroethylamine (Dibenamine): This solution was prepared by dissolving 5 mg. in 0.1 ml. 95 per cent ethanol made faintly acid (approximately 0.05 N) with sulphuric acid and then diluted to 5 ml. with normal saline. This substance was not easily obtained but Messrs. Smith, Kline and French kindly supplied a small amount.

Heparin: (B.P.) The "Boots" preparation (200 u per 100 g. body weight) was employed made up to 0.5 ml. in normal saline.

Urethane: The British Drug Houses preparation was used, 25 g. being dissolved in 100 ml. of distilled water. 175 mg. per 100 g. body weight was injected subcutaneously into the rats.

Vasopressin: International Posterior Pituitary Lobe standard, 100 m.u./ml. of normal made up to 1 ml. normal saline was used/

used as a control substance.

Solution Angiotonin: This was supplied by Dr. O.M. Helmer, of Eli Lilly Research Laboratories, U.S.A. when no source of the substance was available in this country. But for his assistance this section of the work would not have been possible. The substance arrived in solution containing 10 cat units per ml. and was diluted in normal saline or plasma to a strength of 2 cat units per ml. for assay purposes.

Sodium Thioglycollate: This was prepared by adding 0.35 ml. of thioglycollate acid to 0.42 g. of $NaHCO_3$ in 4 ml. of distilled water and making up to 5 ml. when effervescence had ceased. Sodium thioglycollate solutions of vasopressin, angiotonin and plasma to a total concentration of o.1 or o.o1 molar and the mixtures left for varying periods before testing.

Microburette: A 2 ml. microburette with reloading thistle funnel attachment was employed. This delivered accurate volumes of normal saline rapidly and repeatedly into the femoral vein, via fine polythene tubing.

Manometer: The success of the method depended largely on the manometer employed. This was designed and built by N.E. Condon, The Department of Physiology, University of Edinburgh. It consists basically of a U tube with a broad bulb at one side, so that the surface area of mercury therein is infinitely great compared to the capillary tubing on the recording side. The/

The upper part of the bulb contains normal saline which is in direct, air-free contact with the intra-arterial blood of the rat. The capillary tube has a float and on top of this a stilette. By this device the sensitivity of the recording apparatus is doubled and a direct reading of pressure increase obtained, as opposed to the conventional U tube manometer (Fig. XIV).

Method of Assay

The stock solutions having been prepared as above, the albino rat received a subcutaneous injection of urethane. When suitably anaesthetised a median incision was made in the neck and a polythene tracheotomy tube inserted (Fig. XIV and Fig. XV). The carotid artery on one side was dissected clear, three ligatures passed loosely round the vessel and the wound covered with a warm saline pack. The right femoral vein was then exposed, the deep branches ligated and a fine drawn polythene cannula leading from the microburette and containing normal saline was tied into the vein. 200 units of heparin per 100 g. of rat were immediately injected intravenously through a rubber cuff attached to the cannula and washed in with 0.2 ml. of saline from the burette. The cannula having been firmly tied in place, the wound was closed.

The carotid artery was ligated as far distally as possible and the proximal part clamped by a fine arterial clamp. Using iridectomy scissors a small incision was made in the vessel wall and

a/

FIGURE XIV

Method of Bioassay

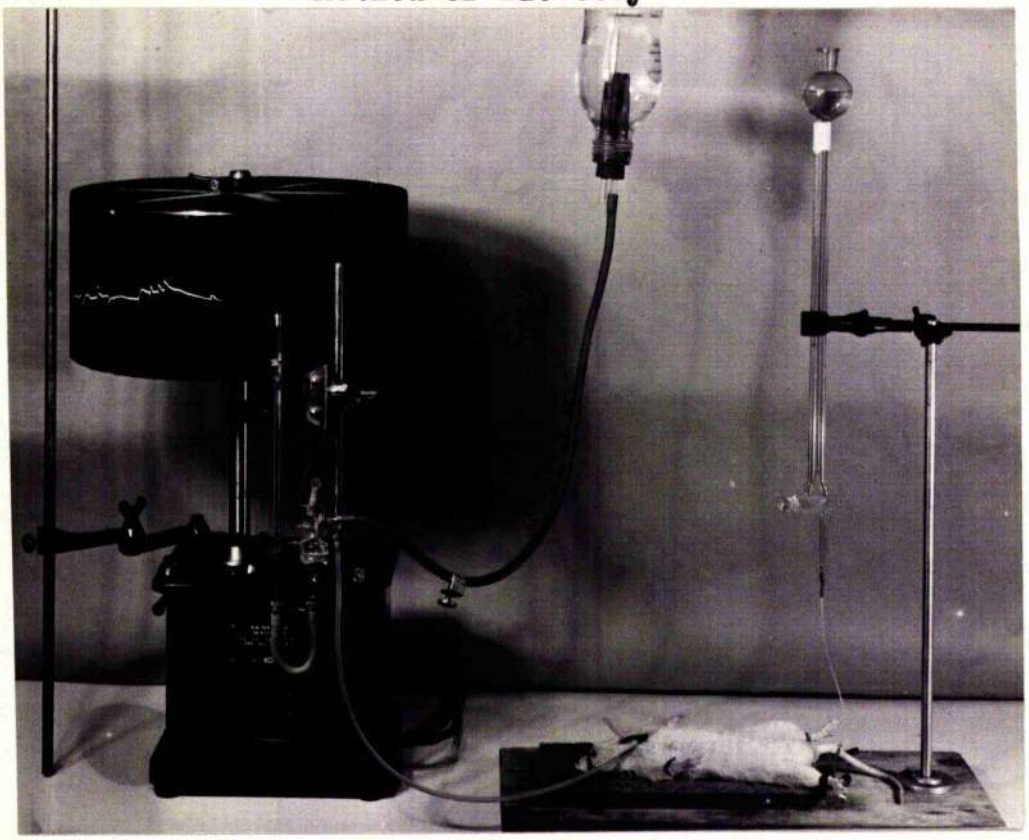
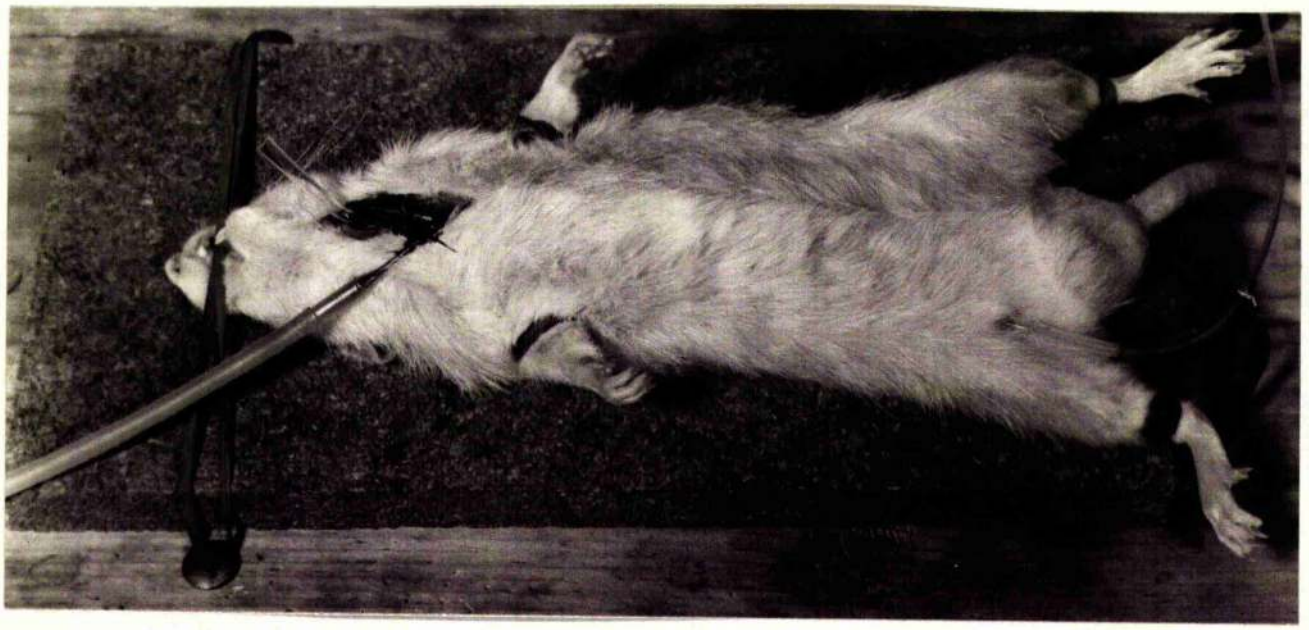


FIGURE XV

Rat Preparation in Detail



a drawn polythene cannula containing normal saline and in direct continuity with the manometer was inserted and tied in place with two ligatures. The saline was kept flowing slowly but continuously to preclude the introduction of air bubbles into the hydraulic system with resultant loss in sensitivity. The tube running from rat to manometer was then connected with the reservoir to make the hydraulic pressure in the system approximately equal to that in the rat's arterial circulation and thus prevent blood loss. The arterial clamp was then released and the stilette at once began to oscillate with the pulse of the rat.

Successive doses of dibenamine (each of 0.5 ml. of the standard solution) were given until the blood pressure settled at about 50 mm. of mercury. The rat preparation was then tested by intravenous injection of standard solution and of normal saline. The work of testing unknown solutions could then commence. After each injection through the rubber collar the dose was washed in with 0.2 ml. of normal saline from the burette.

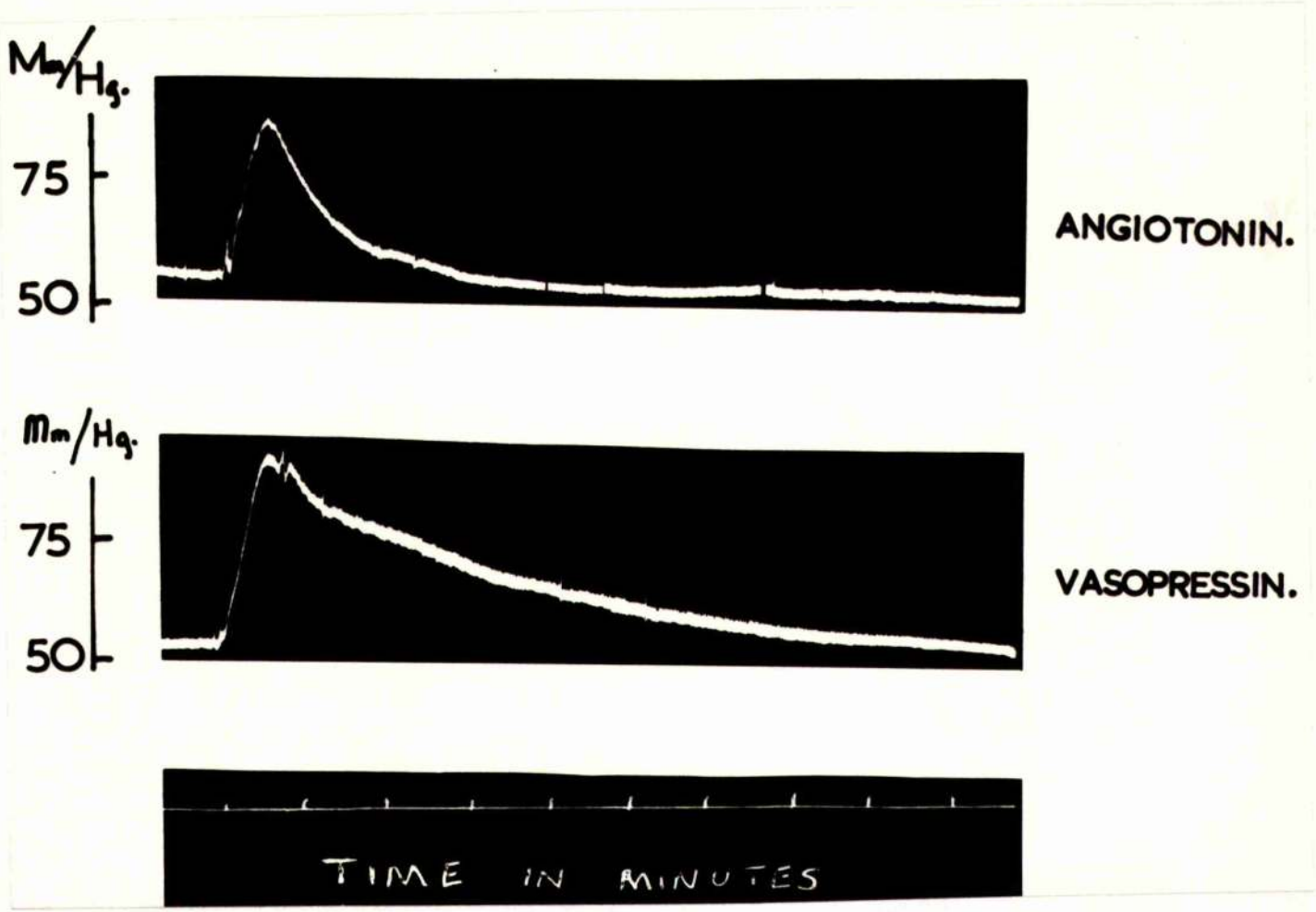
Preliminary Observations

When Solution Angiotonin was injected into the prepared rat it became obvious that a rise in blood pressure resulted. This reaction was compared with standard pituitary vasopressin and the following observations were made:-

(a) The response to Solution Angiotonin was more rapid and much more transient than that of vasopressin (Fig. XVI).

FIGURE XVI

Pattern of Pressor Responses to Angiotonin and to Vasopressin.



The upper part of the tracing shows the response to 0.4 cat units angiotonin; the hypertension is largely over after two minutes. The level is basal in three minutes. The response to 8 milli-units of vasopressin, on the other hand, persists for seven minutes. These are continuous tracings.

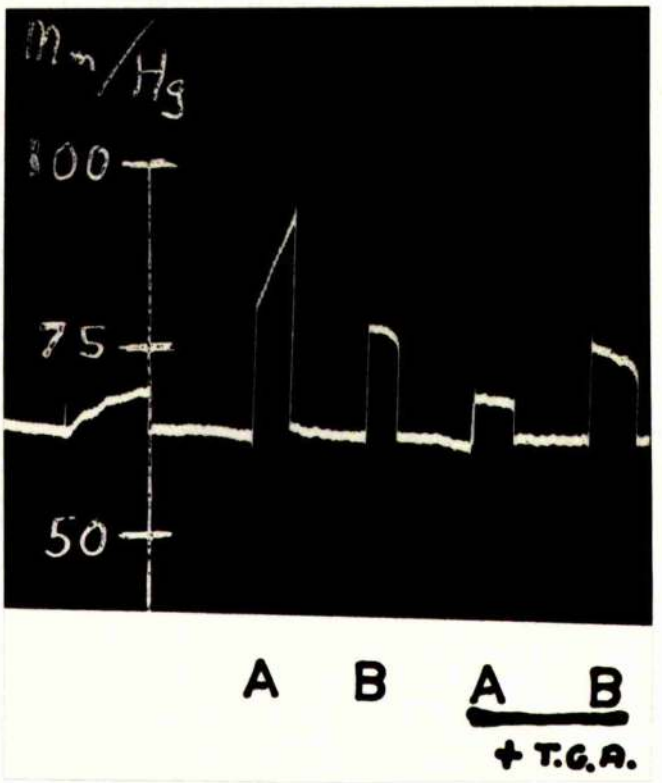
(b) The pressor effect of Solution Angiotonin was not altered by standing for one hour after sodium thioglycollate had been added to 0.01 molar. The pressor effect of vasopressin was abolished by this treatment (Fig. XVII, Fig. XXVIII and XXIX).

Successively larger doses of Solution Angiotonin were then injected sequentially into the rat. The intrinsic order of such series of injections was usually varied but Figure XVIII shows a simple arithmetic progression in dosage. The volume of all injections was equal. From this tracing a straight line graph has been drawn relating the response (rise in blood pressure expressed in mm. of mercury) to the log-dose. During the course of such experiments it became clear that tachyphylaxis did not occur, up to at least 30 injections. These observations were then utilised to provide a four point quantitative method of assay based on the methods of calculation suggested by Holton (1948) for vasopressin. A series of groups of injections was given, each group consisting of a large and a small dose of the standard and the unknown solution. In each case the large dose was twice the potency of the small and all injections were made up to a standard volume with normal saline. Three to five minutes elapsed between each injection, and the group of injections was repeated four times, varying the intrinsic order in each group (e.g. ABDC, ACDB, DCAB, CDBA) as shown in Figure XX. The values for the elevation in blood pressure resulting from such an injection, expressed in mm. of mercury were then obtained.

The graphic results of such a four point assay are shown in Figure/

FIGURE XVII

Pressor Responses to Angiotonin and Vasopressin with and without Added Sodium Thioglycollate.



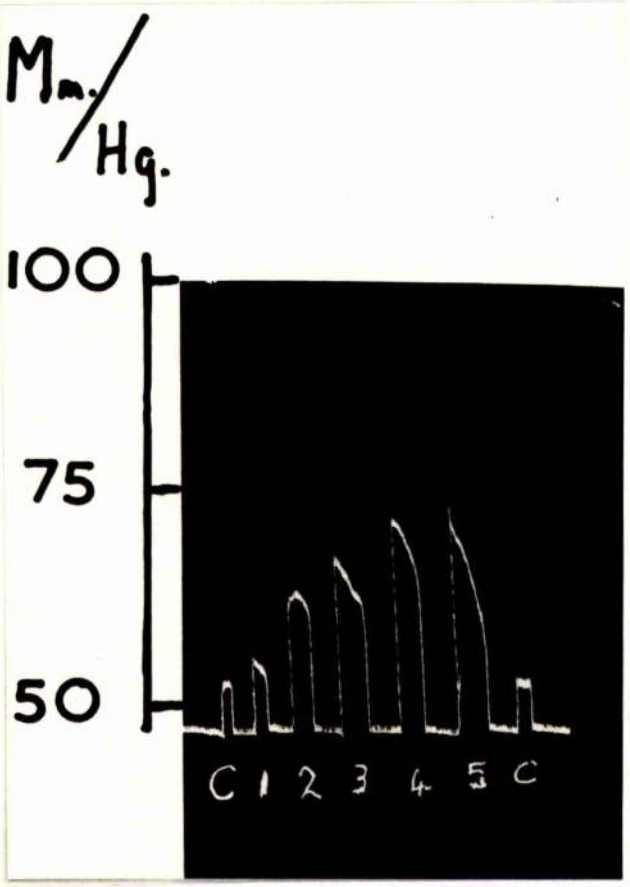
A = 8 milli-units vasopressin

B = 0.2 cat units solution angiotonin

+ T.G.A. = following retention at 0.1 molar sodium thioglycollate for one hour

FIGURE XVIII

Pressor Responses to Increasing Dosage of Angiotonin.

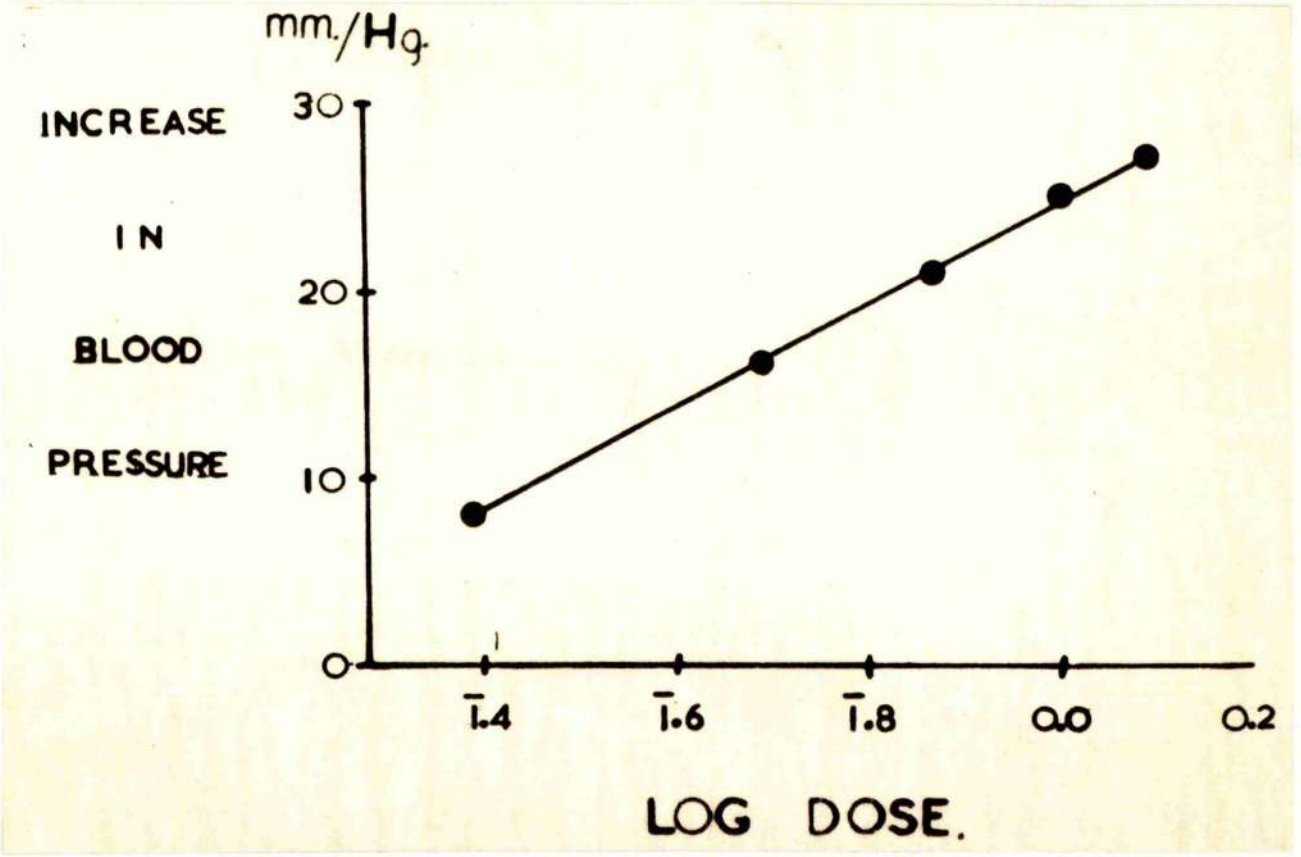


C = 0.4 ml. normal saline

1, 2, 3, 4, 5 = 0.1, 0.2, 0.3, 0.4 and 0.5 cat units of angiotonin made up to 0.4 ml. with normal saline.

FIGURE XIX

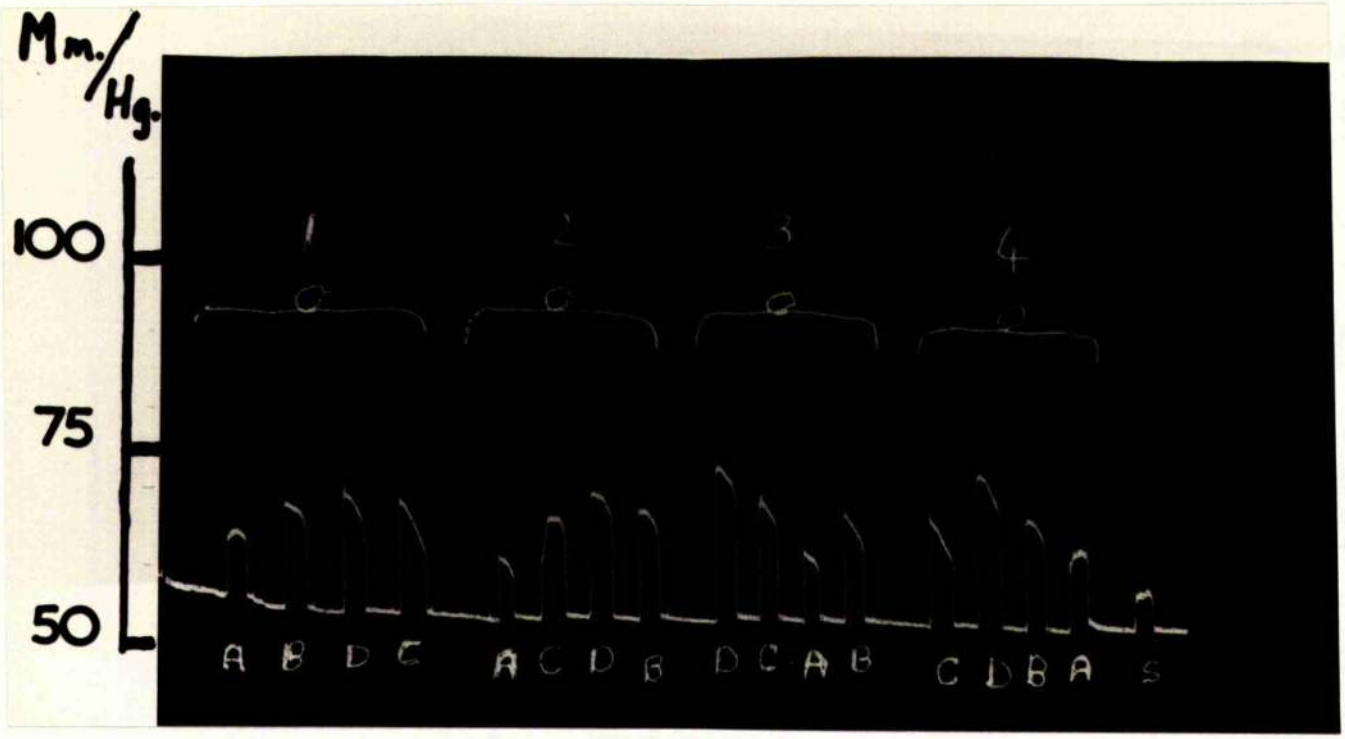
Relation of Pressor Responses (mm./Hg.) to the Logarithm of the Dose of Angiotonin Producing Same.



Dosage expressed in Eli Lilly cat units.
Vertical scale refers to rise of blood pressure (mm./Hg.)

FIGURE XX

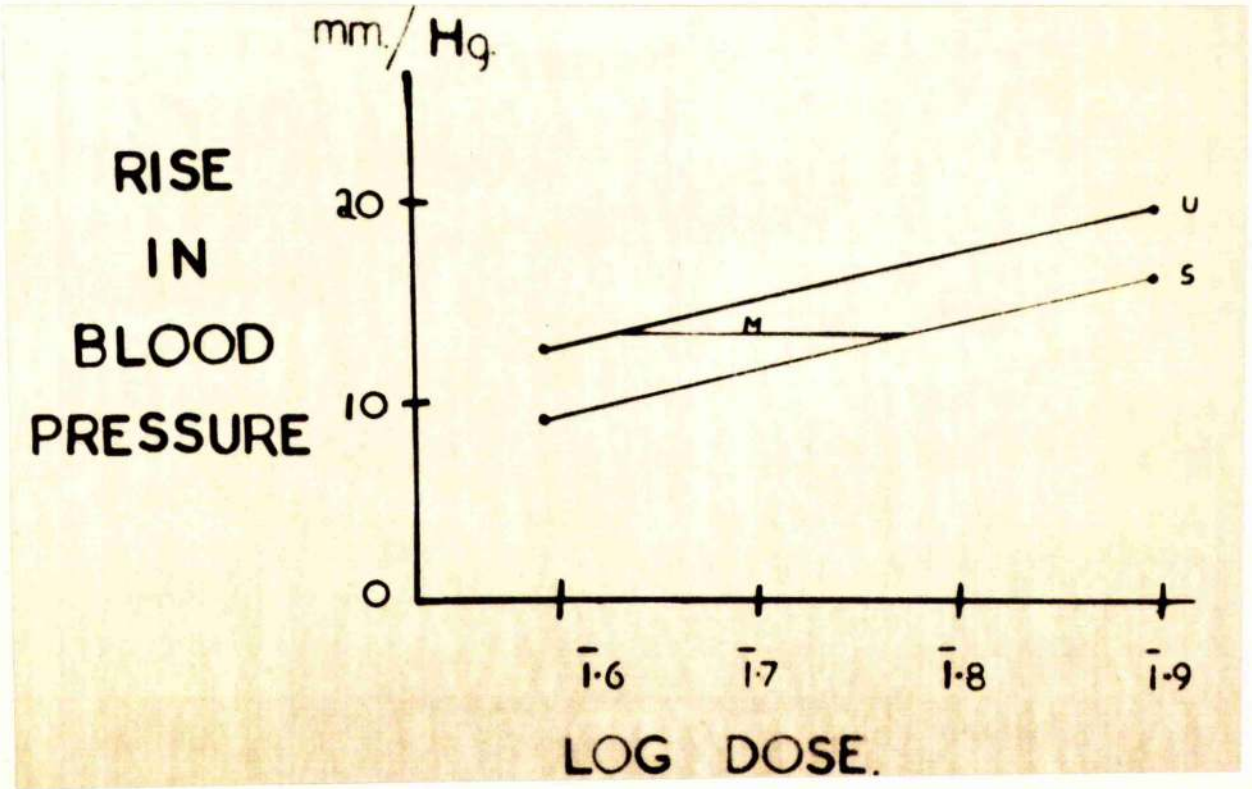
Quantitative Comparison of Unknown and Standard Pressor Solutions.



A & C = 0.4 & 0.8 c.u. angiotonin.
B & D = 0.2 & 0.4 ml. of "unknown "solution.

FIGURE XXI

Graphic Resolution of Results Shown Above.



U = unknown solution. S = standard angiotonin.

Figure XXI. From these data, M (the difference in log-dose of control and standard producing a like vasopressic response) was measured graphically and found to be 0.153. The ratio of standard to unknown = R = antilogarithm M

$\therefore R = 1.422$

Allowing for the volumes concerned the unknown is measured graphically to contain the equivalent of 2.844 cat units of angiotonin per ml. In actual fact this "unknown" solution contained 3 cat units per ml., an experimental error of 5 per cent.

Unfortunately the arithmetical calculation of results is by no means simple. The values obtained from the experiment shown in Fig. XX were as follows:

	Solution	Volume of Injection (ml.)	1	2	3	4	Sum	Mean
A 0.4 c.u.	Standard	0.5	9	8	9	10	36	9
B 0.2 ml.	Unknown	0.5	12	13	13	13	51	12.75
C 0.8 c.u.	Standard	0.5	16	15	17	16	64	16
D 0.4 ml.	Unknown	0.5	19	18	21	20	78	19.5
			56	54	60	59	229	

The statistical methods of Holton (1948) were adopted in the following fashion.

Statistical Methods.

By the null hypothesis (Schild, 1942) since there is no difference between the standard and the unknown substance, standard log-doses/

log-doses may be used in plotting the unknown. The two lines which join the responses to high and low doses of the unknown and standard should therefore be parallel (U & S, Fig. XXI). These are in fact regression lines drawn by eye to be the parallel lines most nearly fitting the points. The regression coefficient "b" is the slope of each line. The line "M" drawn parallel to the base and cutting both regression lines represents the difference in log-doses producing like response. If "b" is the regression coefficient of the parallel lines then :-

$$M = \frac{\overline{Yu} - \overline{Ys}}{b}$$

where $\overline{Yu}$ and $\overline{Ys}$ are the mean responses in blood pressure produced by unknown and standard respectively, where n = the number of injections and

$$b = \frac{\text{sum of responses to high doses - sum of responses to low doses}}{1/2\ n\ \times\ (\text{log. high dose - log. low dose})}$$

Applying these formulae to the results in Table XI

$$\therefore M = 0.158$$

The ratio of unknown to Standard (R) = antilog. M

$$\therefore R = \text{antilog. } 0.158 = 1.44$$

Therefore the ratio of the dose of unknown to that of standard (2 cat units/ml.) is 1.44 to 1.00 and the unknown solution contains the equivalent of 2.88 cat units per ml. It has been seen that in fact this solution contained 3 cat units per ml., the error in estimation being 4 per cent.

Since the ratio of "unknown" to standard was in fact revealed to have been 1.5 : 1 the standard error of M (Sm) may now be calculated, in order to assess the reliability of the method. This may be obtained from the standard deviation of Sy of a single observation (y). This observation, which represents the error in the assay is measured by analysing the variance due to known causes and that due to the variability of the preparation separately. The sums of squares of the deviations attributable to four sources of variation are shown in Table III. The sum of squares of deviations of all the observations from the common mean, which is the total sum of squares, is 251.44 in this example.

When the sum of squares due to known causes have been subtracted from this total sum of squares the remainder is the error/

error of assay. The remainder, divided by its nine degrees of freedom gives the variance of an individual observation according to the usual formula for standard deviation. In this estimation the residual sum of squares is 4.0625. (This has nine degrees of freedom because from (n-1) i.e. 15, 6 must be subtracted to allow for the known causes of variation.)

$$\therefore \text{ Variance, } s^2_y = 0.4514$$

These results are displayed in Table III below in which F represents the variation ratio, e.g. for the deviation from parallel

$$F = \frac{0.0625}{0.4514}$$

TABLE III

Source of Variation	Sum of Squares	Degrees of Freedom	Variance	F	P
Between groups	5.6875	3	1.8958	4.20	$.01<p<.05$
Between unknown and standard	52.5625	1	52.5625	116.4	<0.001
Between high and low dose (regression)	189.0625	1	189.0625	418.8	<0.001
Deviation from parallel	0.0625	1	0.0625	0.14	$>.20$
Error of assay	4.0625	9	0.4514	-	-
Total sum of squares	251.4375	15	-	-	-

A table (Fisher and Yates, 1948) of F for $n_1 = 1$ and $n_2 = 9$ (where/

(where n_1 and n_2 are the degrees of freedom for deviation from parallel and error variance respectively) gives $p > 0.20$. This means that there is a probability of high degree that this amount of deviation would occur by chance and so the slopes do not differ significantly; similarly the values given in column P refer in each instance to the probability of the results having arisen by chance. Thus there is less than one chance in one thousand that the difference between unknown and standard is fortuitous or that the regression slopes are not significant.

The standard error of M may be obtained by applying Schild's formula:-

(d = (log. high dose - log. low dose)).

$$S^2M = \frac{4S^2y}{nb^2}\left\{\frac{M^2}{d^2} + L\right\} = 0.0002765$$

$$SM = \sqrt{0.0002765} = 0.0165$$

The 95% confidence limits of M are $M \pm$ (Sm x t) where t is a value obtained from tables by the degrees of freedom applicable to the error sum of the squares. In this example it is t = 2.262.

$\therefore$ Fiducial limits of M = $0.158 \pm 0.0375 = 0.120 - 0.196$

R = antilog. M $\therefore$ limits for R = 1.322 - 1.572

These calculations depend on the relationship of log-dose and pressor response being exactly linear. This is probably true only within a limited range. In practice values from 0.10 to 1.00 cat units were found to be a satisfactory range of dosage for this rat preparation.

The methods employed and results obtained closely accord with those parallelly obtained by Dekanski (1954). The sensitivity of

the/

the method appeared quite satisfactory and vasopressic activity of 0.1 cat unit could easily be differentiated. Since this was much more delicate measurement than any previously described it seemed possible that it might prove of value both in the standardising of solutions and in measuring much smaller amounts of activity than had hitherto been possible. This hope proved short-lived for in 1954 Picarelli and co-workers described a much easier method which would discriminate activity less than one twentieth of a cat unit. If the claims for this guinea-pig ileum method are substantiated it will doubtless be preferred, since it is so very much easier to perform. It is ironical that an almost identical technique to this had been qualitatively employed by me during the course of this work in the differentiation of angiotonin and activated plasma (see later) but not used for quantitative estimations.

DEVELOPMENT OF VASOPRESSIC ACTIVITY IN PLASMA FROM NORMAL SUBJECTS.

During the course of these investigations it had been noted on occasions that plasma derived from apparently normal subjects possessed unexpected and unexplained vasopressic activity when tested in the dibensamised rat (Arneil and Dekanski, 1954). An attempt to investigate the source of such activity was undertaken as follows.

Methods

Ten male and ten female subjects aged from 7 to 40 years and in good/

good health were selected. Systemic venous blood was removed by syringe and 100 units of heparin (Boots) added to each millilitre of blood. The blood was spun immediately at 1500 revolutions per minute for fifteen minutes and the supernatant plasma removed and chilled to 4°C. During the course of various experiments this plasma was incubated; on such occasions incubation was at 37°C and the plasma was therafter chilled to and retained at 4°C as before. The vasopressic activity of these plasma samples was tested by injection into the dibenamised rat, using the methods described in the foregoing chapter.

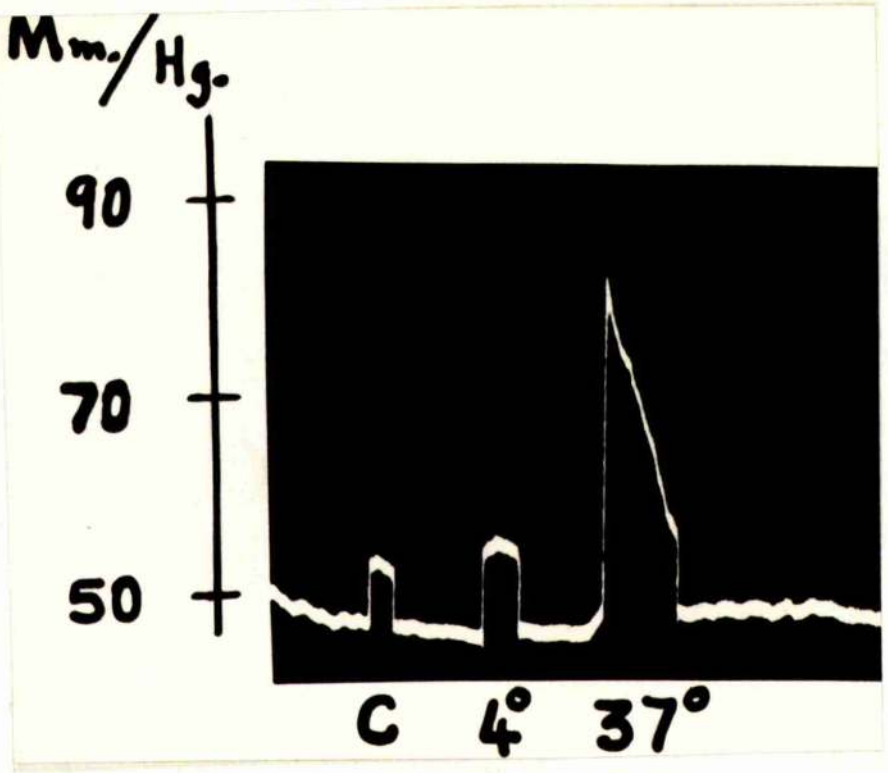
Results

The plasma from each of the twenty normal subjects was divided into two parts: one portion was chilled to 4°C at once and the remainder retained at 37°C and then cooled as before. In each instance the plasma which had been kept cold possessed little more vasopressic activity than an equivolumetric injection of normal saline whereas that which had been incubated possessed marked vaso-pressic activity. Qualitative illustrations of this happening are shown in Figures XXII and XXIII. Quantitative measurement was essayed using Solution Angiotonin; this proved unreliable (for reasons given later) and only approximate mensuration could be made. Nevertheless it may be stated with confidence that the plasma from each of these twenty subjects acquired a level of vasopressic activity considerably in excess of the equivalent of 100 cat units of angiotonin (Lilly) per 100 ml.

An/

FIGURE XXII

Pressor Responses to Saline and to Normal Plasma Retained at 4°C and 37°C for 12 hours Respectively.

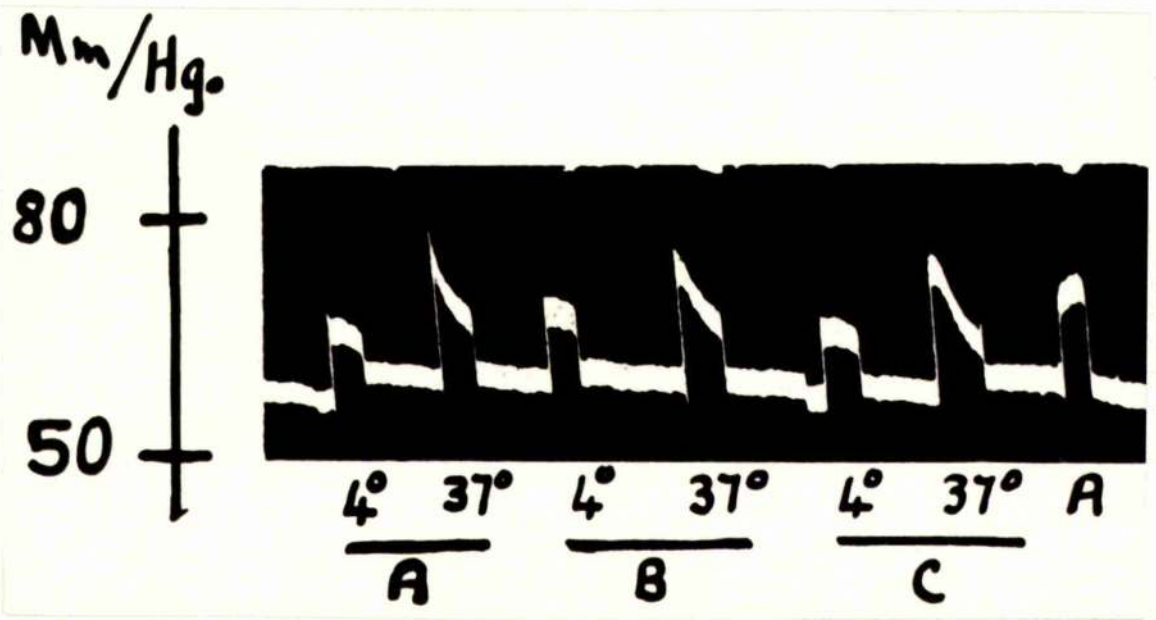


C = 0.4 ml. normal saline

4°, 37° = 0.4 ml. of "normal" plasma prev. retained at temperature indicated for 12 hours

FIGURE XXIII

Pressor Responses to Further Samples of Plasma Retained at 4°C and 37°C for 12 hours.



A), B), C) = Plasma from 3 normal controls

A' = 0.4 c.u. angiotonin

This tracing derives from a 300 g. rat which proved much less sensitive to the vasopressic agent than the 240 g. rat used in Figure XXII.

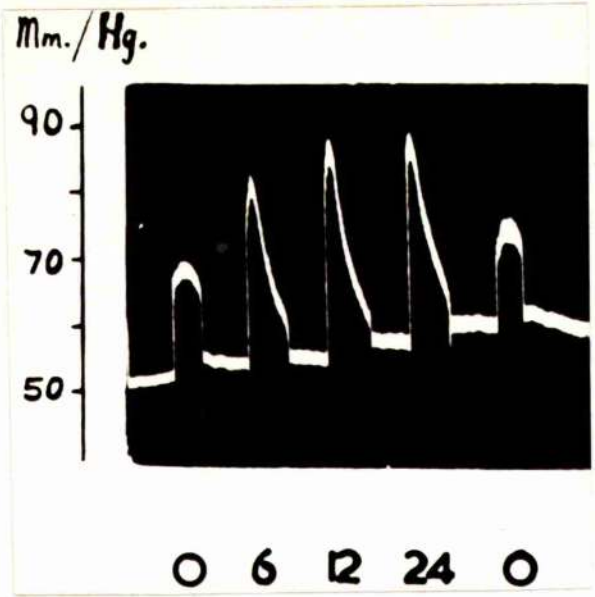
An attempt was then made to assess the rate at which such vasopressic activity was gained and further samples of plasma were obtained and incubated for varying periods. Figures XXIV and XXV illustrate the results obtained in two typical cases; activity was marked within six hours, maximal by twenty-four hours and when incubated for longer periods activity diminished rather than increased. These findings were confirmed in six samples of plasma. It was noted that plasma could be incubated and then chilled, or chilled and then incubated, or chilled, incubated and then chilled once more and yet retain its acquired vasopressic activity. This property persisted even after many months at $4^{\circ}C$.

When sequentially larger amounts of activated plasma were injected into the rat the resultant alterations in blood pressure occurring are shown on Figure XXVI. Graphically these results suggest a straight line relationship between rise in blood pressure (mm./hg.) and log-dose (Fig. XXVII) such as might result from the presence of a pharmacologically active substance.

The cause of such development of vasopressic activity was unknown and no account of comparable findings were to hand. The substances most likely to be responsible seemed to be the adrenalines, the organic amines, the vasotonins, pituitary vasopressin or hypertensin. The rat preparation used excluded the action of some of these substances. Thus pressor activity could not have been due to adrenaline, nor-adrenaline, iso-amylamine, tyramine, piperidine or nicotine/

FIGURE XXIV

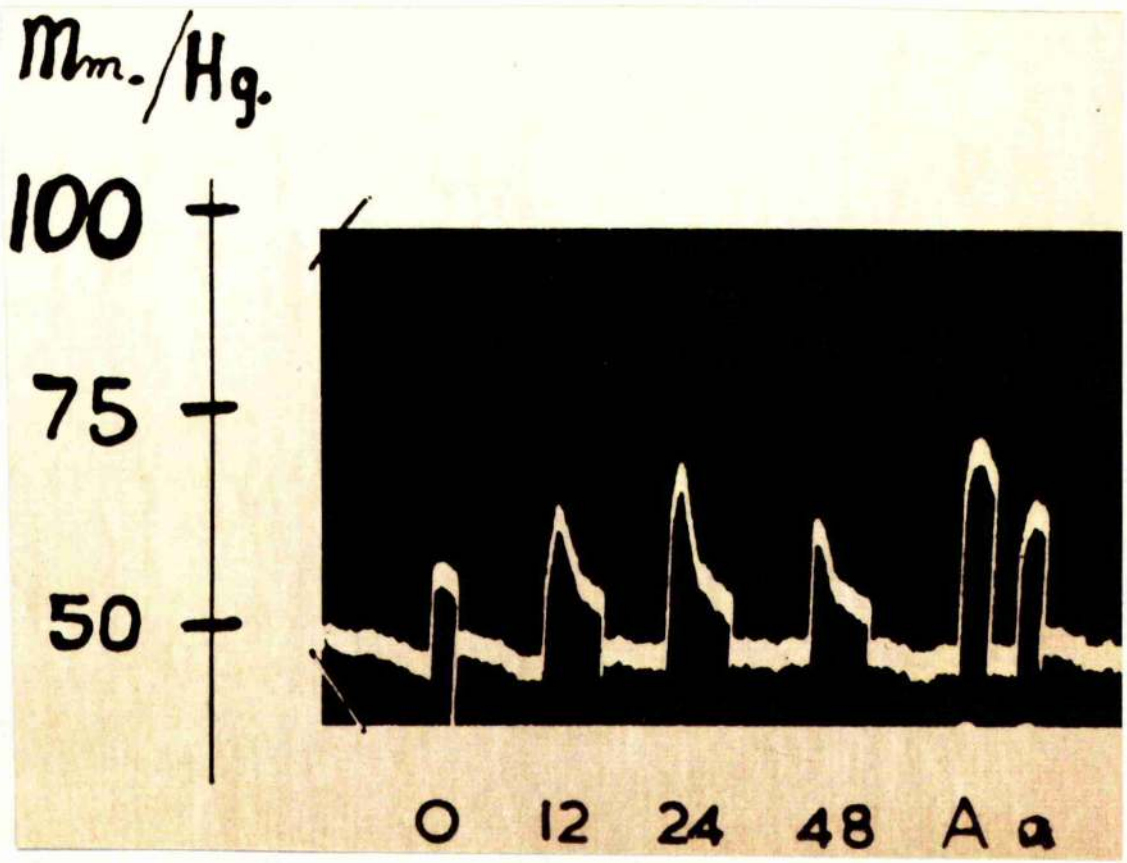
Pressor Responses to Plasma Stored at 37°C for Varying Periods.



0) refer to injection
6) of 0.4 ml. of
12) normal plasma
24) retained at 37°C
for 0, 6, 12 & 24
hours respectively.

FIGURE XXV

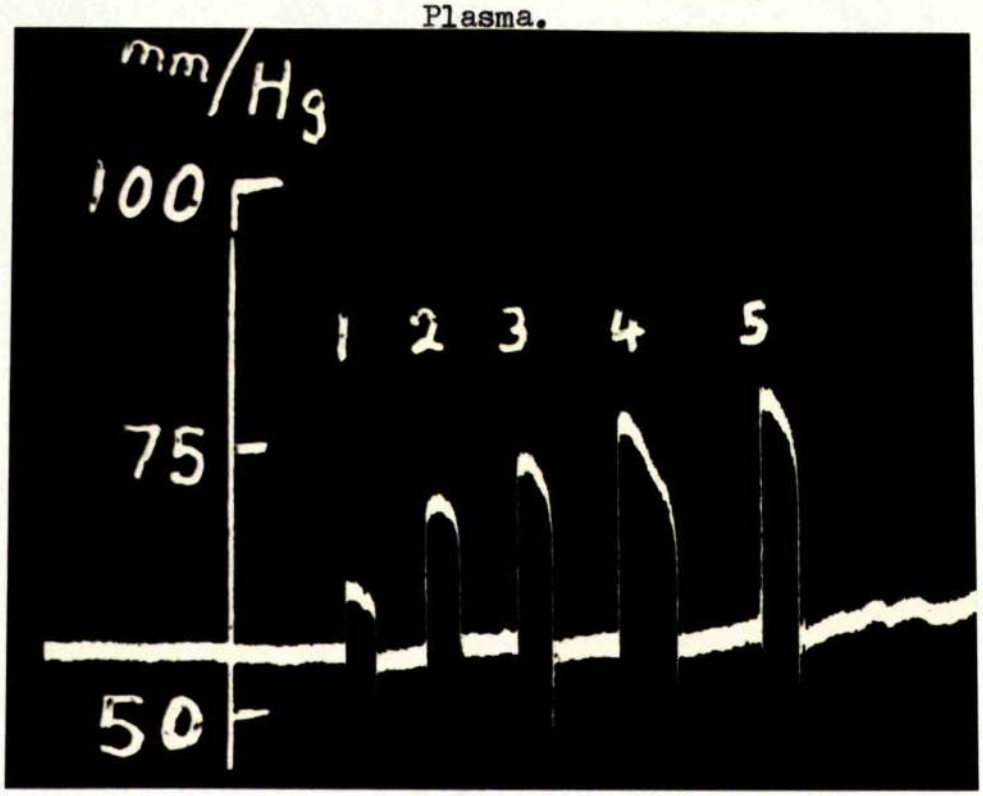
Pressor Responses to Plasma Stored at 37°C for Varying Periods.



0, 12, 24, 48 = no. of hours at 37°C before injection of 0.4 ml. plasma.
Aa = 0.8 & 0.4 c.u. solution angiotonin respectively.

FIGURE XXVI

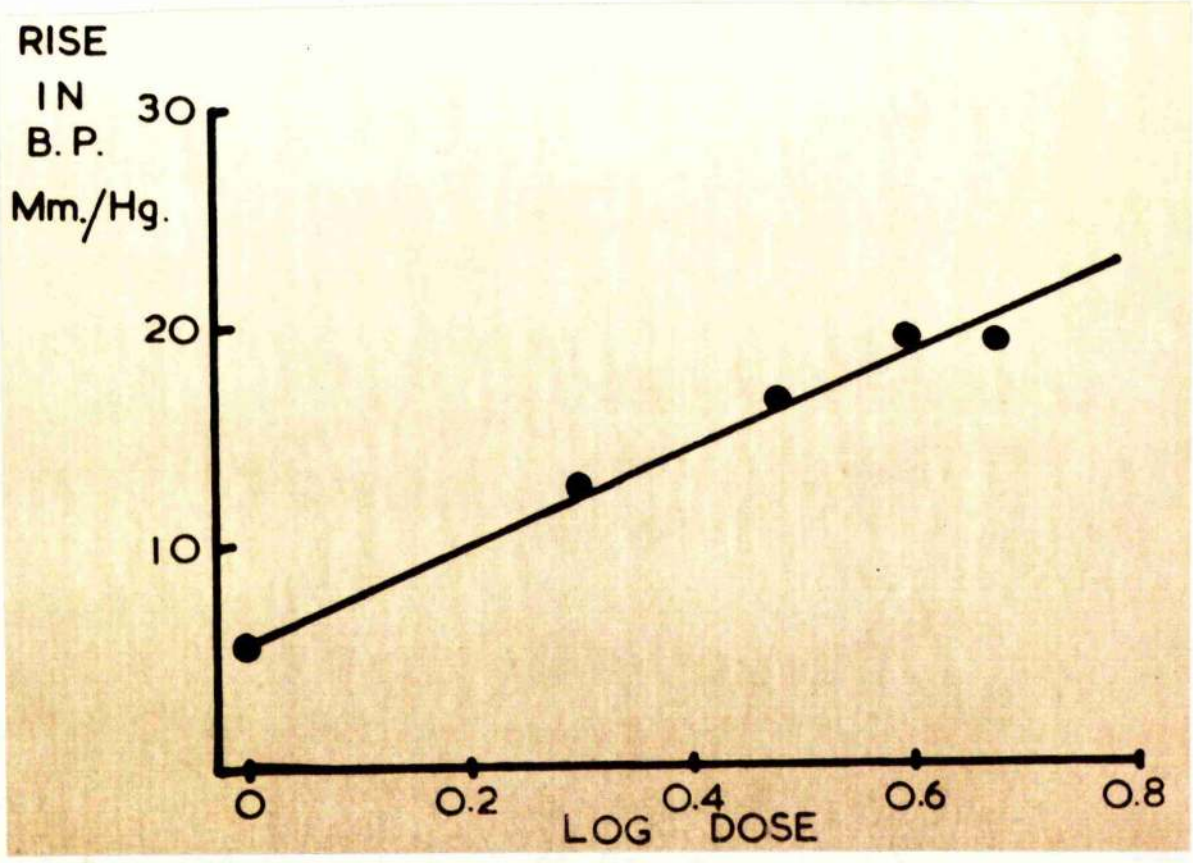
Pressor Responses to Injections of Increasing Volumes of Active Plasma.



1, 2, 3, 4, & 5 refer to injection of 0.1, 0.2, 0.3, 0.4 and 0.5 ml. of plasma respectively.

FIGURE XXVII

Graphic Resolution of Results Shown Above.



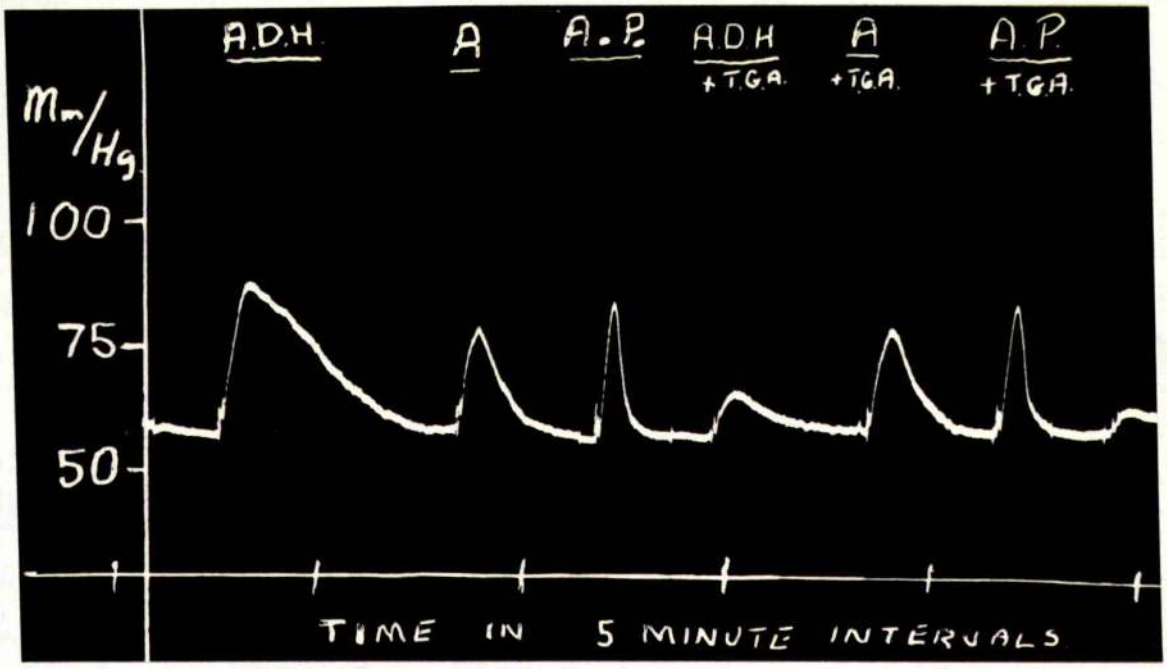
nicotine, since the action of these are blocked by dibenamine (Dekanski, 1951, 1952). Furthermore, the vasotonins ((of which serotonin (5-hydroxytryptamine) is the most important)) have a depressor effect on the blood pressure of the dibenamised rat and should therefore mask rather than produce pressor effect (Dekanski, 1954). The pressor effects of pituitary vasopressin and the active plasma factor were then compared. Differences were at once apparent. The duration of the pressor response to vasopressin in saline or in plasma was much longer than that of active plasma (Fig. XXVIII) and prior treatment to 0.01 molar with fresh sodium thioglycollate for 30 minutes virtually abolished the activity of vasopressin whilst the activity of plasma was not affected materially (Fig. XXVIII and Fig. XXIX). For these reasons it seemed that plasma pressor activity was not due to pituitary vasopressin.

As in the case of plasma taken from hypertensive patients with acute nephritis it was considered possible that the substance concerned might be hypertensin derived from the enzymatic action of renin on hypertensinogen, which is greater at 37°C than at 4°C. When careful comparison was made however several minor differences came to be noted.

The first of these was a difference in the character of the elevation of blood pressure produced. When active plasma is injected the rise in blood pressure is very rapid, a sharp peak is followed by an immediate fall with a shouldering effect as the pressure nears normal levels once more. This characteristic appearance is seen to advantage/

FIGURE XXVIII

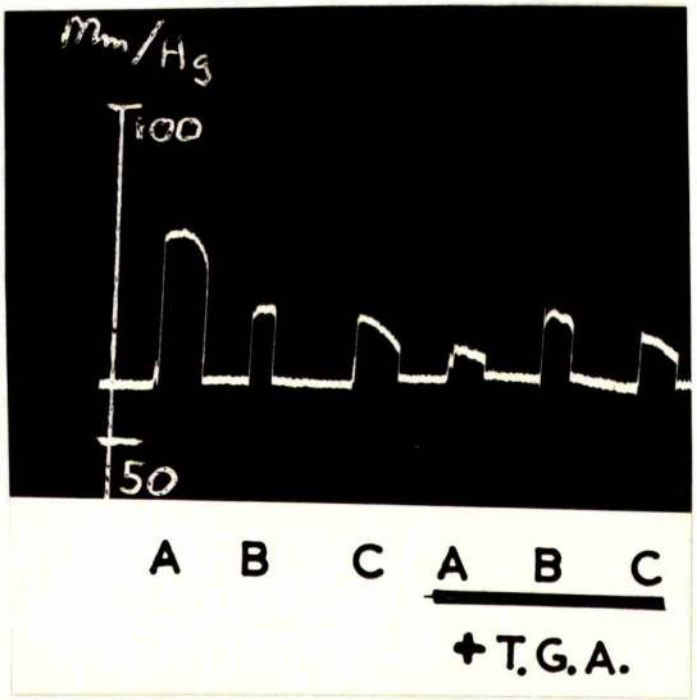
Pressor Responses to Vasopressin, Angiotonin and Active Plasma + 30 Minutes Treatment to 0.01 Molar with Sodium Thioglycollate (Continuous Tracing).



ADH = 8 milli-units vasopressin in plasma
A = 0.4 c.u. angiotonin in plasma
AP = 0.4 ml. plasma after 12 hours at 37°C

FIGURE XXIX

Pressor Responses to Vasopressin, Angiotonin and Active Plasma.



A = 8 milli-units vasopressin
B = 0.4 c.u. angiotonin
C = 0.4 ml. plasma after 6 hours at 37°C

\+ T.G.A. indicates prior treatment to 0.01 molar sodium thioglycollate for 30 minutes

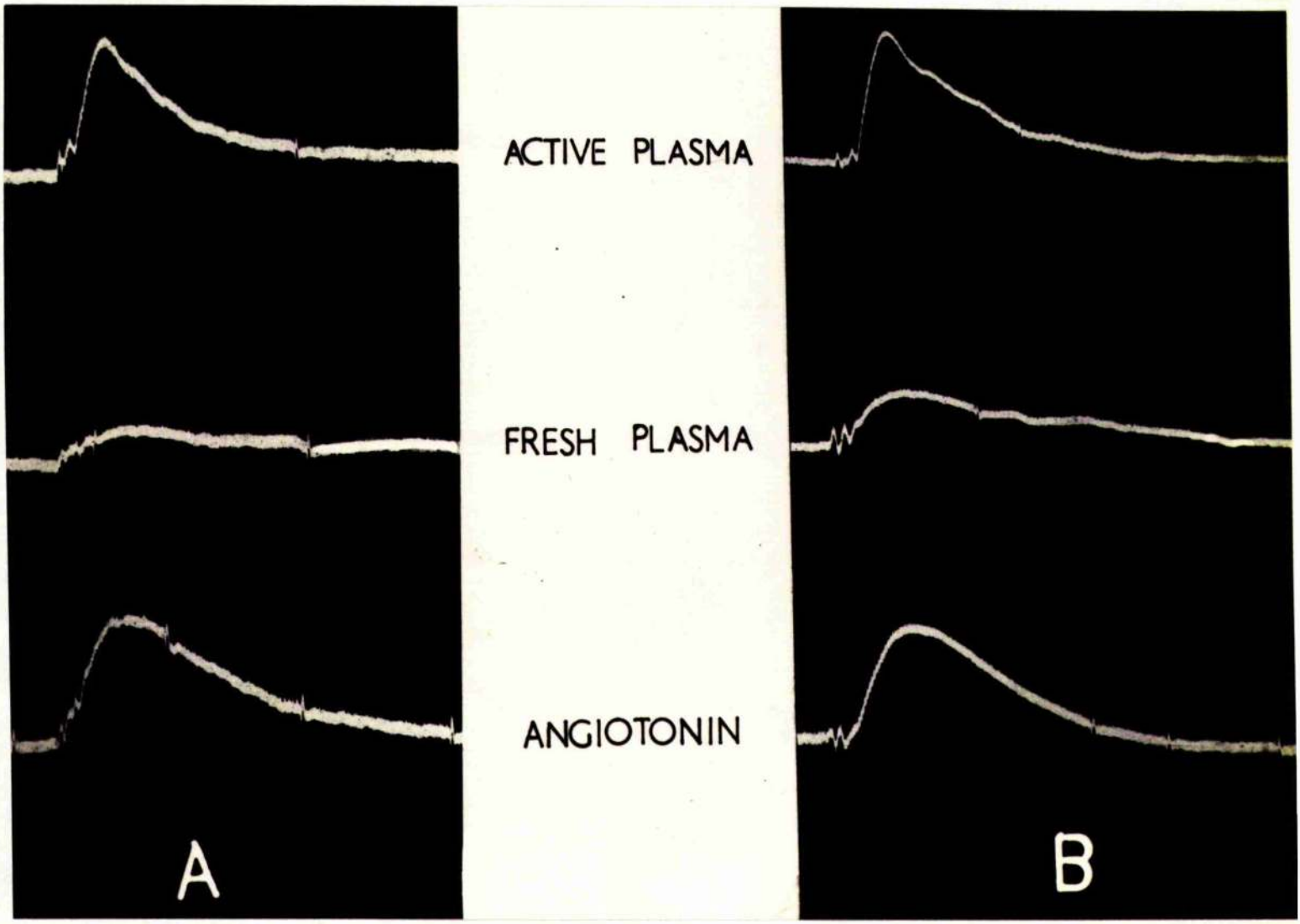
advantage in Figures XXIII, XXIV and XXV. When angiotonin is injected the rise is less rapid and the high level maintained for a longer period before the steady fall to the baseline ensues (Fig. XXV and Fig. XXXII.) These impressions of difference were gained when employing the standard method of recording alterations in blood pressure (i.e. with the drum stationary during the rise and during the latter part of the fall). By this method more readings may be recorded in a shorter length of tracing. When this difference in pattern was being studied it was decided to compare continuous tracings and this has been done in Figures XXVIII, XXX and XXXI. The difference of the pattern of response to active plasma and angiotonin is quite clear. With a little experience the pattern of these pressor responses may be recognised visually with comparative ease. (See Fig. XXXII).

The second observation pointing to a difference existing between the two substances was the fact that the relative sensitivity of the rat to these two substances seemed to vary from time to time and animal to animal. The fortuitous results of errors in technique emphasised this point as follows. Incubated plasma was being compared in activity with angiotonin when air embolism occurred, a not unusual mishap. Before this accident the rat had been responding well to both vasopressic substances but following the incident reacted to angiotonin and not to active plasma (Fig. XXXII). A similar occurrence was noted on two subsequent occasions.

Thirdly/

FIGURE XXX

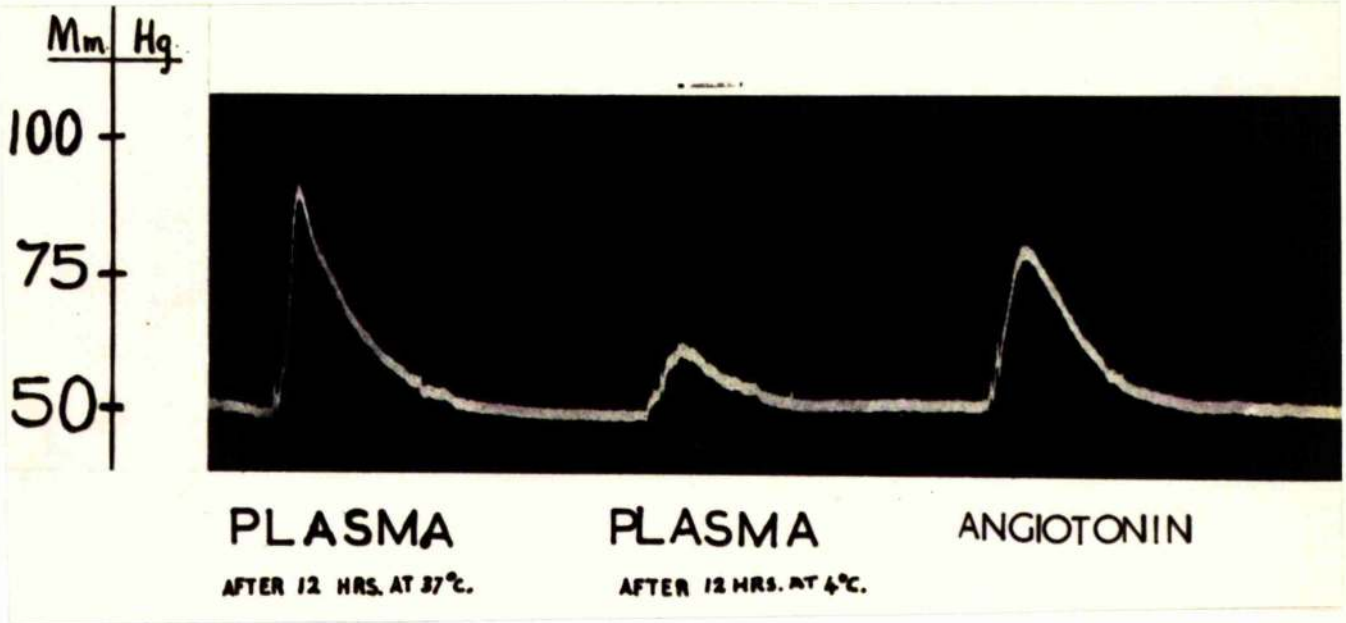
Pattern of Pressor Responses to Plasma Previously Retained at 37°C for 12 hours, Fresh Plasma and Angiotonin.



Each injection was of 0.4 ml. The dosage of angiotonin was 0.4 c.u. A and B refer to two different rat preparations; note the close similarity of responses. In each case the response to active plasma is more sharply peaked and more evanescent than that to angiotonin (see also Figures XXXI and XXVIII).

FIGURE XXXI

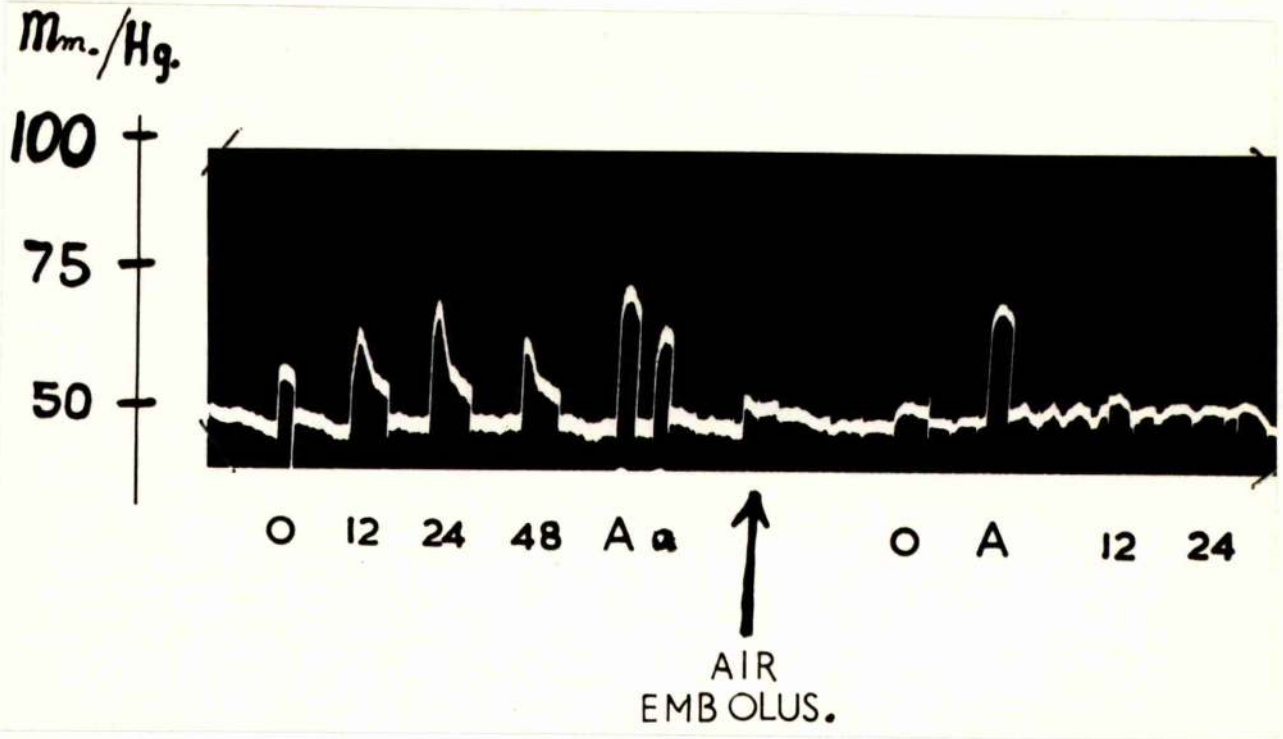
Pattern of Pressor Responses to Plasma and to Angiotonin.



All volumes = 0.4 ml. The dosage of angiotonin used was 0.4 cat units. The sharp peak and "concave" fall in blood pressure after injection of active plasma is well seen. It is this characteristic of the fall in blood pressure which produces the characteristic shouldering (see Figure XXXII).

FIGURE XXXII

Pressor Responses to Active Plasma and Angiotonin Before and After Air Embolism.



10 ml. of fresh plasma was divided into four parts, retained at 37°C for 0, 12, 24 and 48 hours respectively, and then chilled to 4°C. 0.4 ml. of each sample was injected and then 0.4 and 0.2 c.u. of angiotonin. At this time air embolism occurred and subsequently the injections of "0", "12", "24" and of 0.4 c.u. angiotonin were repeated.

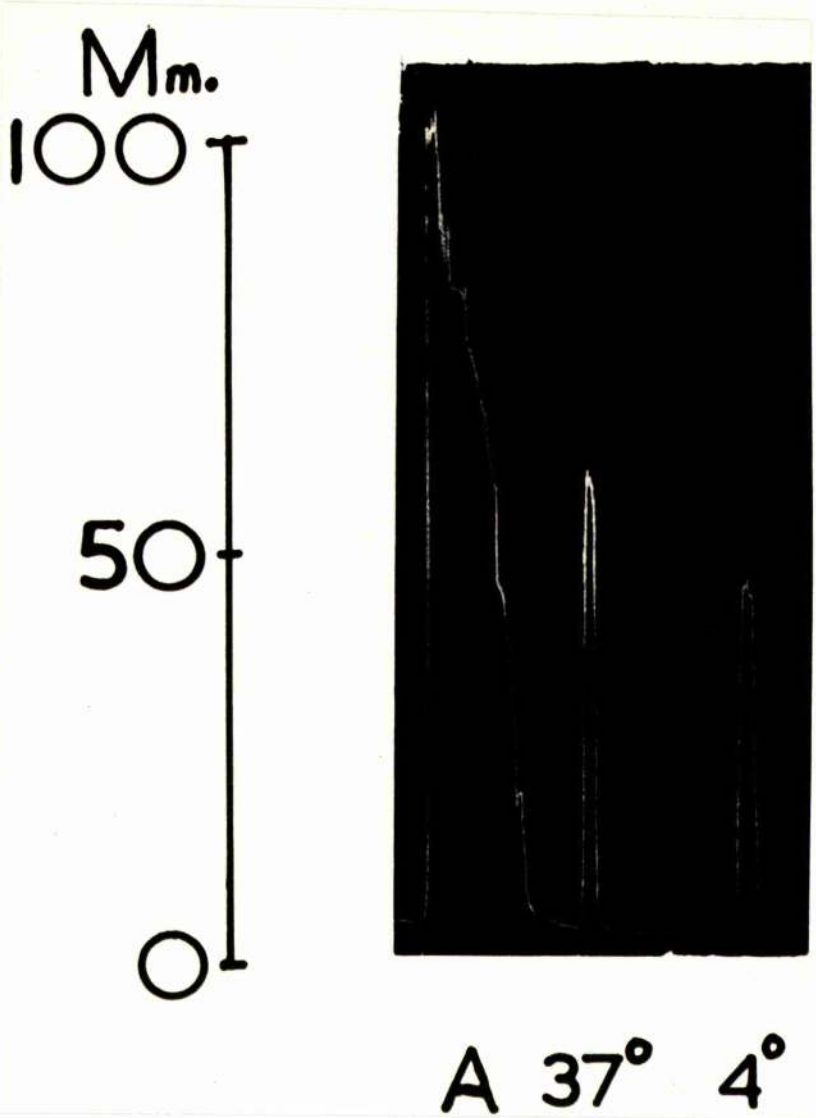
Thirdly, following the suggestion of Gaddum (1955) that, when in doubt about the identity or otherwise of two factors, they should be compared using a different method of assay. Angiotonin and active plasma were compared using the isolated guinea-pig ileum preparation in Tyrode's solution. The pattern of contraction was quite different when these two substances were injected (Fig. XXXIII). The contraction produced by angiotonin was much greater and of longer duration than that resulting from a dose of activated plasma which would have produced an approximately equal rise in blood pressure in the rat preparation. Furthermore, the contraction of the ileum was not much greater when incubated plasma was used than when fresh plasma was employed.

From these three findings it seems likely that this substance is not identical to angiotonin. It is possible that human hypertensin might vary slightly from that of lower vertebrates but this seems a very speculative hypothesis. For the same reasons angiotonin is not a reliable standard by which to measure this plasma vasopressic factor and any such quantitative mensuration should be accepted with reserve.

The work of Reid and Bick (1942a and b) drew attention to a factor present in fresh serum and old plasma which they found caused contractions in the isolated ox carotid artery and guinea-pig uterus. They considered this substance to be derived from platelet disintegration. Experiments were carried out to compare the/

FIGURE XXXIII

Response of Isolated Guinea Pig Ileum to Perfusion with Various Fluids.



A = 0.4 c.u. angiotonin.
37°, 4° = 0.4 ml. plasma from normal subject retained for 12 hours previously at 37°C and 4°C respectively.

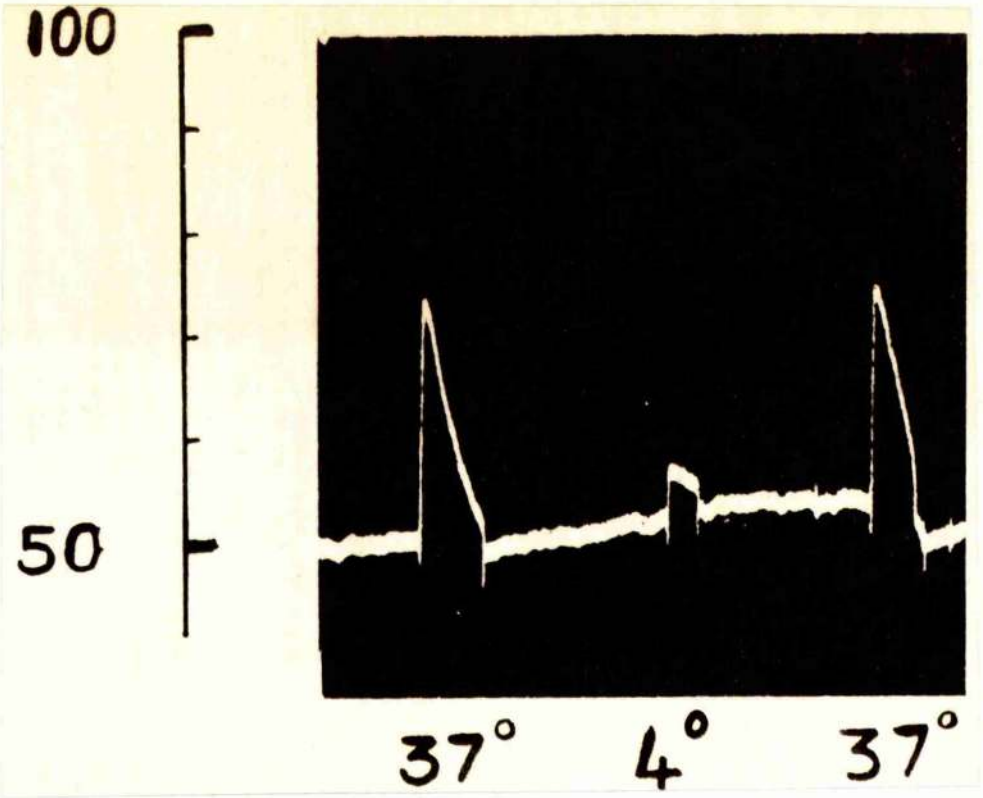
the effects of the plasma pressor substance and the factor described by Reid and Bick in serum. Blood was withdrawn and divided into four parts: the first was allowed to clot and the serum removed; the second was heparinised and centrifuged at 3,000 rev. for 30 minutes to remove thrombocytes; from the third portion plasma was removed by the standard method; and the fourth specimen received treatment similar to that accorded to the third but was shaken vigorously with glass beads for 30 minutes to encourage platelet disintegration.

No excess of vasopressic activity in the serum was noted until it had been warmed to 37°C (Fig. XXXIV) for a period of hours. The thrombocytopenic plasma developed vasopressic activity on incubation as before (Fig. XXXV). The fourth specimen possessed no excess of vasopressic activity until incubated (Fig. XXXVI). It therefore seems that this vasopressic factor is not released during blood clotting, is not dependent on the presence of a large number of platelets, and is not produced when platelets break down in large numbers. For all these reasons it does not seem to be identical to the factor described by Reid and Bick.

It seems therefore that human plasma will regularly develop an excess of vasopressic activity as tested in the dibenamised rat when incubated at 37°C. This activity is not caused by the adrenalines, serotonin, pituitary vasopressin, angiotonin or the factor of Reid and Bick. The exact nature of the substance responsible/

FIGURE XXXIV

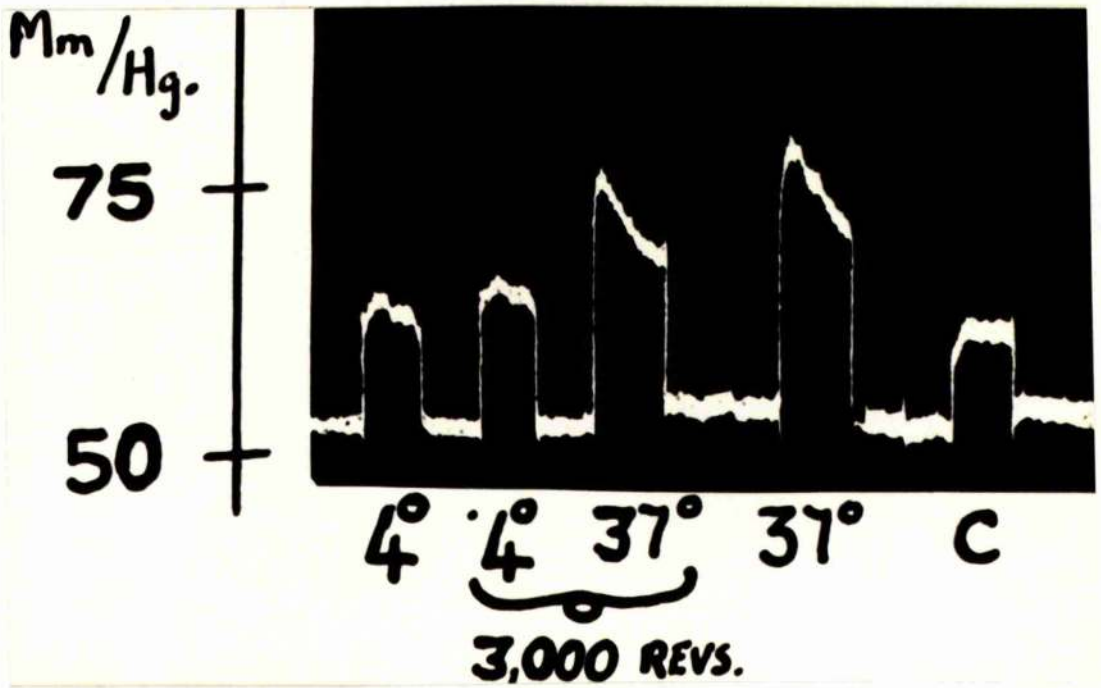
Pressor Responses to Samples of Serum.



Vertical scale refers to blood pressure of rat (mm./Hg.) 4°, 37° refers to the injection of 0.4 ml. of serum retained at 4°C and 37°C respectively for 12 hours prior to injection.

FIGURE XXXV

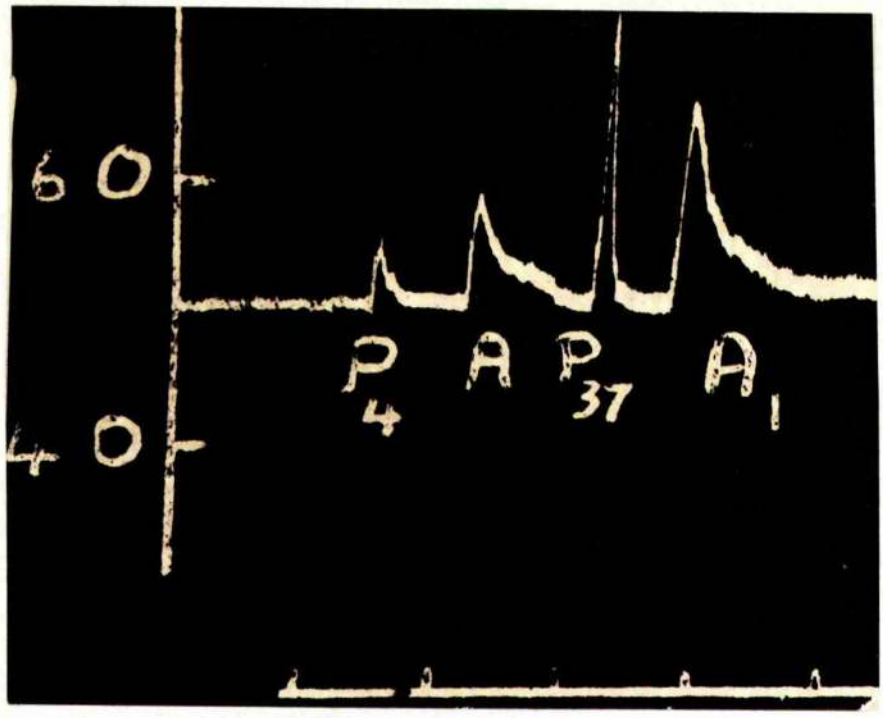
Pressor Responses to Thrombocytonormic and Thrombocytopenic Plasma Samples.



4° and 37° refers to prior retention at this temperature for 12 hours. Each injection = 0.4 ml. plasma. C = 0.4 ml. normal saline.

FIGURE XXXVI

Pressor Responses to Agitated Plasma.



P_4, P_{37} refer to the injection of 0.4 ml. of plasma, previously shaken with glass beads and retained at temperature indicated.

A, A_1 = injection of 0.2 & 0.4 c.u. angiotonin respectively.

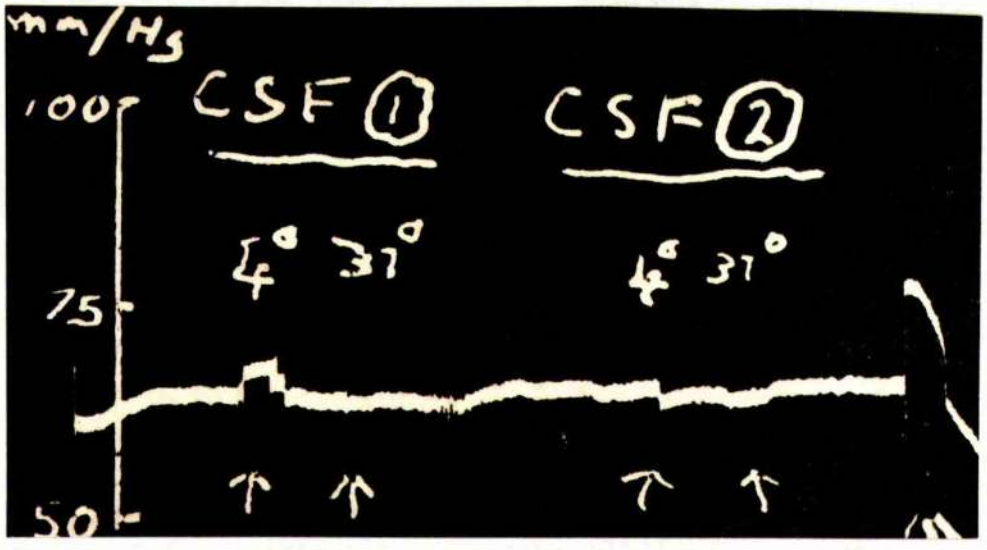
Vertical scale = B.P. in mm./Hg.

Horizontal scale = time in 5 minute intervals.

Continuous tracing.

FIGURE XXXVII

Pressor Responses to 0.4 ml. Cerebro-Spinal Fluid.



4^0 and 37° refer to injections of 0.4 ml. C.S.F. previously retained at this temperature for 12 hours. Final injection = 0.2 ml. of plasma previously retained at 37°C for 12 hours.

responsible is as yet unknown. Cerebro-spinal fluid does not develop this pressor activity when incubated at 37°C (Fig. XXXVII).

EXCESS OF VASOPRESSIC ACTIVITY IN BLOOD "BANK" PLASMA

Since 1829 when the first successful transfusion of human blood was reported in The Lancet by Blundell the use of this valuable therapeutic measure has become widespread. The subsequent use of citrated blood (Lewisohn, 1915) greatly increased the scope of such treatment, permitting indirect transfusion and storage of blood in bank form. Furthermore, this led to the realisation that corpuscles were not necessary to save the life of a man dying of haemorrhage (Ward, 1918) and alternative fluid to whole blood began to be used. Plasma, which required no grouping, withstood storage for longer periods, and was capable of drying and reconstitution, came to be regarded as of great value and proved successful in the treatment of battle casualties, shock, haemorrhage and burns. As more and more rapid rates of transfusion came to be recommended (Graham, 1944, Harkins et al. 1945) some alarm began to be felt at the possibility of deleterious side effects arising from alterations occurring in plasma constitution during storage (Keynes, 1949).

Normal plasma retains the capacity to develop vasopressic activity on incubation indefinitely when retained at 4°C and this seemed to parallel the conditions prevailing when plasma is stored in a blood bank preparatory to transfusion. For this reason samples/

samples of such plasma were obtained from the local blood bank (thanks to the courtesy of the Director) and tested for vasopressic activity.

Details of Material Used

Eight samples of plasma were obtained from the local blood bank. The time which had elapsed between the removal of blood from the donors and vasopressic estimation varied from 70 to 258 days. The plasma had been prepared by a modification of the process suggested by Maizels (1944) which is briefly as follows:-

400 g. of kaolin B.P. is weighed into a wide-mouthed bottle of 5 l. capacity. To this, 400 ml. of distilled water is added and well mixed. The addition of the water is necessary to ensure efficient sterilisation of the kaolin in the autoclave. The flask is closed with a two-holed rubber stopper fitted with suitable glass tubing and is then sterilised at 121°C for one hour. After pressure in the autoclave has been released, 15 inches of vacuum is applied in order to dry the kaolin as much as possible. Plasma (approximately 4 l.) is withdrawn under aseptic conditions, from 16 bottles of blood into each of the kaolin bottles. The pooled plasma and kaolin are well mixed by repeated shaking during 15 minutes and then stored frozen at -10°C for not less than 5 days.

When required for processing, the plasma is allowed to thaw at 4°C. When completely thawed out, it is gently mixed and allowed to stand at 4°C for 24 hours to allow the kaolin to settle. The supernatant fluid is first clarified by passage through asbestos pads of clarifying quality and then sterilised by passage through sterilising asbestos pads in a frame filter. The sterile plasma is filled aseptically, in 400 ml. quantities, into sterile transfusion bottles. The processed plasma is incubated at 20°C in a dark cupboard for 21 days. Before issue each bottle is carefully inspected for the presence of foreign bodies and bacterial or fungal contamination. Plasma prepared in this way is clear and, being practically free from fibrinogen and prothrombin, will not form fibrin clots on storage for periods of about 4 to 6 months.

Results

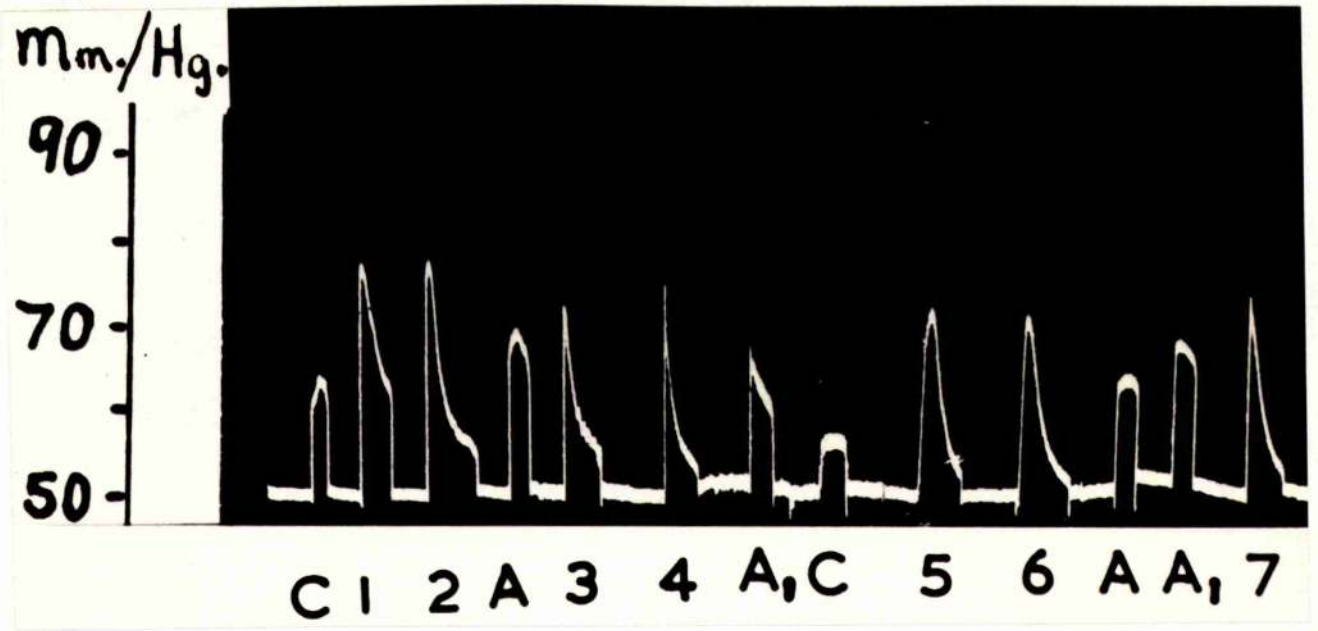
Each of the eight samples of plasma (which varied in age from 70 to 258 days) was found to contain an excess of vasopressic activity when tested in the dibenamised rat. These results are shown qualitatively in Figure XXXVIII. An approximation of the quantity of pressor activity present revealed a range of from 120 - 210 cat units per 100 ml. plasma. It will be seen that the pattern of pressor response produced by the blood bank plasma is identical to that arising when fresh plasma is retained at 37°C for some hours. It seems likely that the vasopressic activity develops in part during the period of 21 days when the plasma is retained at a temperature of 20°C prior to bottling and this supposition is supported by the experimental results shown in Figure XXXIX.

The vasopressic concentration obtaining in these samples of plasma are very considerable in degree. It seemed possible that plasma administered rapidly as an intravenous drip might produce a pharmacological rise in the blood pressure. The hypothesis was tested experimentally in the following fashion. An intravenous drip was set up on a dibenamised rat whose blood pressure was being continuously recorded. Fresh plasma was then infused at a rate of 0.25 ml. per minute for a period of four minutes. The animal was rested for ten minutes and the intravenous drip was repeated for a similar time, at the same rate using bank plasma. Figure XL illustrates the results, showing

clearly/

FIGURE XXXVIII

Pressor Responses to Injection of Various Samples of Blood Bank Plasma and of Angiotonin.



1, 2, 3, 4, 5, 6, 7, refer to injection of 0.4 ml. of various samples of blood bank plasma with <u>no</u> previous incubation at 37°C.

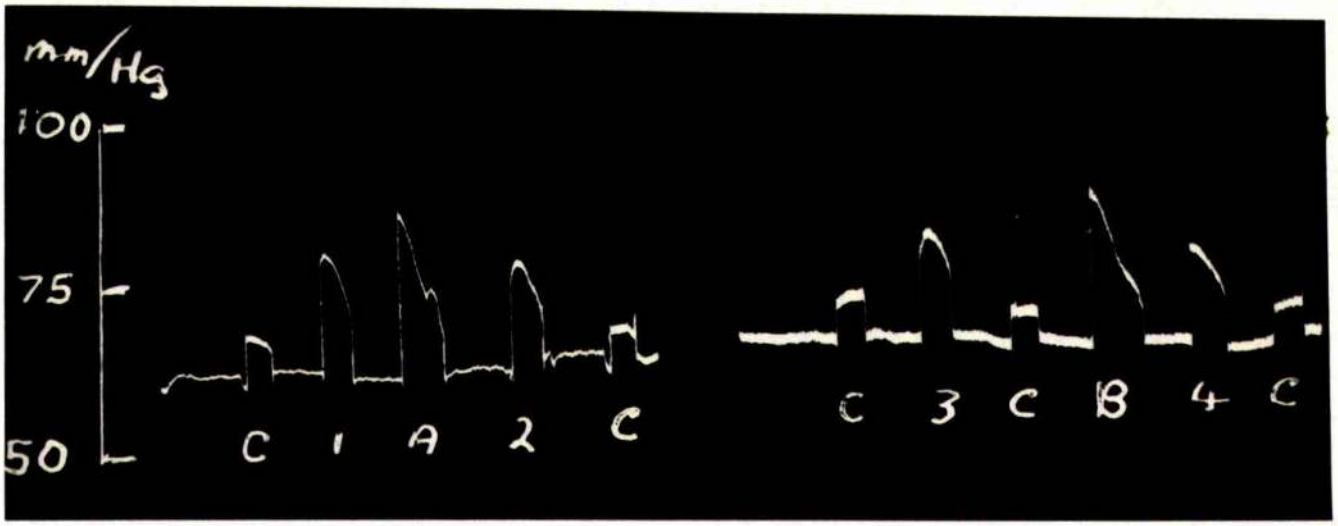
C = 0.4 ml. fresh plasma.

A, A1 = 0.4 c.u. angiotonin from one of two control solutions (A and A_1).

Note that the sensitivity of the preparation lessened slightly during the course of this experiment.

FIGURE XXXIX

Pressor Responses to Various Samples of Blood Bank Plasma



C = Injection of 0.6 normal saline

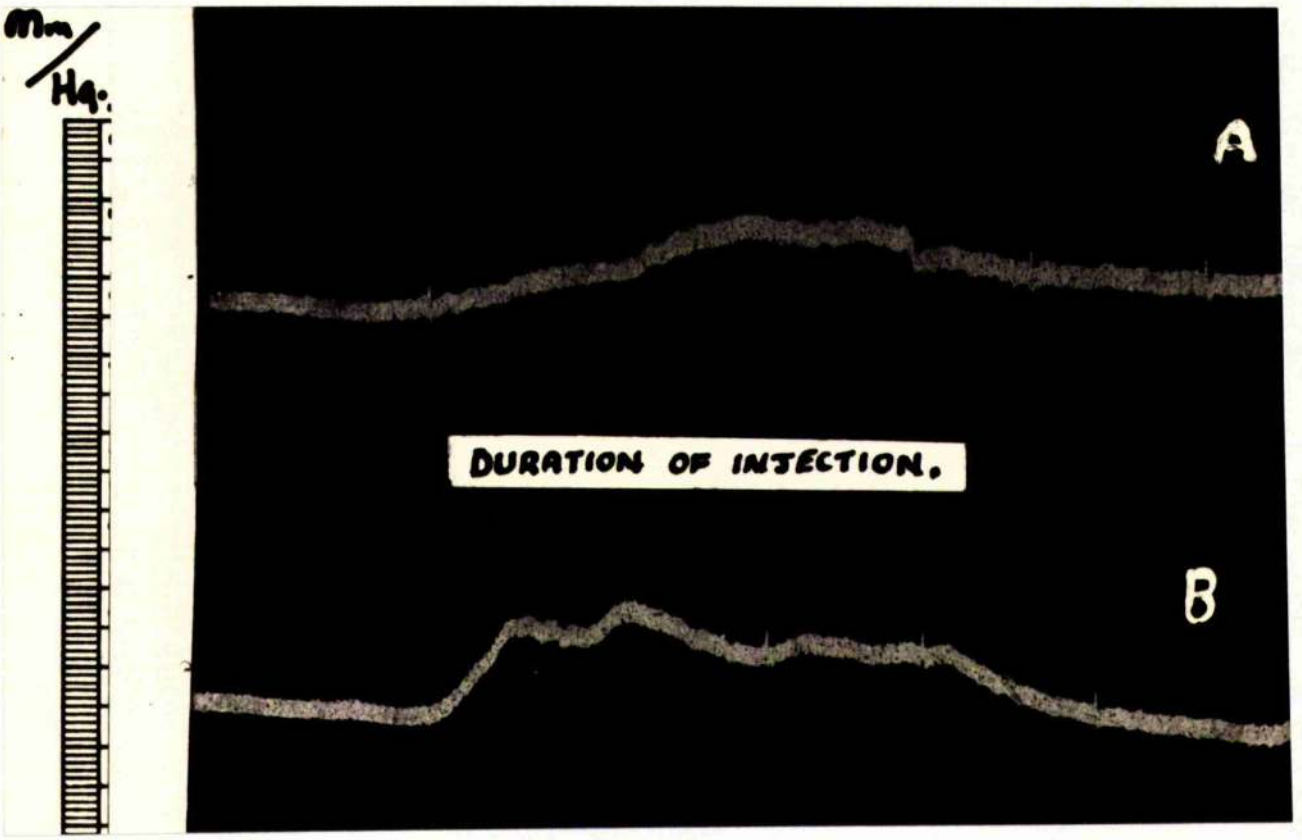
1, 2, 3, 4 = Injection of 0.4 ml. of plasma prepared by blood bank prior to retention at 20°C during processing.

A & B = Injection of 0.4 ml. of plasma prepared by blood bank after retention at 20°C during processing.

Note that the plasma (1, 2, 3 & 4) possesses considerable vasopressic activity before being retained at 20°C. Samples 1, 2 and A are derived from one "pool" of plasma and samples 3, 4 and B from another.

FIGURE XL

Pattern of Pressor Response to Continuous Intravenous Drip of (A) Fresh Plasma and (B) Blood Bank Plasma.



Duration of infusion = 240 seconds

Volume of infusion = 1 ml.

clearly that the blood bank plasma causes a more rapid rise to a higher level than fresh plasma. Such a rate of administration approximates to the administration of one pint of plasma to the adult human in 15 minutes (i.e. 35 ml. per minute). This rate is much slower than the 143 ml. per minute advocated by Graham (1944) and others (Keynes, 1949). It would seem, that if man is as sensitive to this plasma factor as rat, then the rapid administration of plasma may be accompanied by a pharmacological alteration of the pattern of the rise in blood pressure. No continuous method of observing human blood pressure is as yet sufficiently accurate to make possible the direct measurement of this phenomenon if such in fact occurs. It would superficially appear that a vasopressic property might in fact be of benefit to shocked and hypotensive patients. Despite this benefit the fact that a fluid which is frequently introduced in large quantities intravenously into very ill patients contains a pharmacologically active substance whose exact propensities have not as yet been defined is a matter which must give rise to some concern.

It would seem prudent in the light of this finding that the question of examining the exact properties of stored plasma should be raised once more.

CONCLUSION

A pressor substance develops in human plasma when it is retained

at/

at 37°C for a few hours or at lower temperatures for a longer period. This substance is unknown, but is not vasopressin, adrenaline, noradrenaline, angiotonin, a vasotonin or a known vasopressic organic amine. It is present in excess in blood bank plasma, and occurs more freely in the plasma of nephritic subjects with hypertension. Various properties of the pressor activity are described.

PART II

A SURVEY OF 164 CHILDREN WITH NEPHROSIS

"The living organism does not really exist in the milieu extérieur (the atmosphere if it breathes, salt or fresh water if that is its element) but in the liquid milieu intérieur ..... the stability of which is the primary condition for freedom and independence of existence: the mechanism which allows of this is that which ensures the milieu intérieur the maintenance of all the conditions necessary to the life of the elements."

Claude Bernarde

INTRODUCTION

The syndrome characterised by albuminuria and hypoproteinaemia is readily recognised clinically and is called nephrosis. This term nephrosis used by Müller (1905) has also been employed by other investigators such as Volhard and Fahr (1914), Munk (1918), Capon (1926), Evans (1932), Farr (1942), Galan (1949), Allen (1952), Fishberg (1954) and Todd (1957). Unfortunately in each instance a slightly differing interpretation has been placed on the meaning of the word. Other synonyms such as tubular nephritis (Wolbach, 1930), lipoid nephrosis (Block, 1948 and Schwartz, 1943), lipaemic nephrosis (Heymann, 1946) and oedematous nephritis (Rennie, 1947) have also been employed. Ellis (1942) explained the nephrotic syndrome as a second form of nephritis but this over-simplification did little to elucidate the problem and gave an impression of the syndrome as occurring almost entirely in adults and having a very high mortality rate, when in fact the syndrome is commoner in children and relatively benign. The term "the protein losing kidney" used by Platt in 1959 also refers to this state.

Since so many synonyms are extent it is imperative that the individual investigator should define the meaning of nephrosis as used by him. The term "nephrotic syndrome" is used to refer to all conditions in which the clinical picture of considerable proteinuria/

proteinuria, hypo-albuminaemia, oedema and hyperlipaemia co-exist. The underlying disorder may be one of a variety of diseases and various causes of the syndrome may be outlined thus.

THE NEPHROTIC SYNDROME

(1) Infantile Nephrosis: A familial disease, probably congenital and always fatal, whose occurrence is being recognised with increasing frequency.

(2) Toxic Nephrosis: Proteinuria following ingestion of a drug such as gold salts, or tridione, or a poison such as corrosive sublimate and hypersensitivity to bee stings or poison oak.

(3) Collagen Nephrosis: This arises during the course of disseminated lupus erythematosus, anaphylactoid purpura and polyarteritis nodosum.

(4) Renal Vein Thrombosis: This sometimes presents as nephrosis.

(5) Symptomatic Nephrosis: Associated with metabolic processes such as amyloidosis or diabetes mellitus, infections such as syphilis or neoplasia such as diffuse sarcomatosis.

(6) Post-Nephritic Nephrosis: A very rare condition following some time after an attack of acute glomerulo-nephritis and usually with impaired glomerular function co-existent.

(7) "Nephrosis": This represents the bulk of all cases occurring during childhood in which no obvious cause is apparent. Throughout this communication the unqualified term "nephrosis" is applied to cases of the nephrotic syndrome which did not fall into any one of groups 1 - 6.

****

Material

The children were admitted to the Royal Hospital for Sick Children, Glasgow, during the period 1929 - 1957. Their medical care throughout this period was under the supervision of one of three senior paediatricians giving a reasonable degree of continuity and comparability of diagnostic criteria throughout. Children with the nephrotic syndrome and falling into groups 1 - 6 as described above have been excluded and the four cases of infantile nephrosis are described elsewhere.

There remain 164 children who were admitted with nephrosis. Each one of these cases has now been traced. Ninety-one of the 102 survivors have been examined here and the remaining 11 examined on my behalf at various clinics in England, Africa, Asia, Australasia and the Americas. Sixty-two patients have died and the medical practitioners responsible for their care and for certifying the causes of death or carrying out post mortem examinations (where applicable) have been interrogated, or the relevant reports scrutinised. Reasonably accurate assessment of the cause of death has been possible in each case.

The hospital records of these patients are extensive and give a detailed history of each patient and the results of the physical examination on admission to hospital carried out by a junior and a senior paediatrician. Where possible the daily volume of urine passed by each patient was recorded together with the/

the protein content therein (Esbach method). Patients were weighed regularly and the blood pressure recorded by sphygmomanometry. Biochemical investigation included routine investigation of the non-protein nitrogen (N.P.N.) level in blood and the serum protein level. Fractionation of serum proteins was carried out by chemical means (after Howe 1921) and/or electrophoresis (after Cremer and Tiselius, 1950). Blood cholesterol levels were measured by chloroform extraction in 144 children (Lieboff, 1924). Detailed analysis of the biochemistry of urine and serum has been carried out by chromatography, bio-assay, electrophoresis, spectrophotometry and various other methods, (Arneil and Wilson, 1952, 1953a and b, Arneil 1956a, b and c, 1958, 1959).

Findings

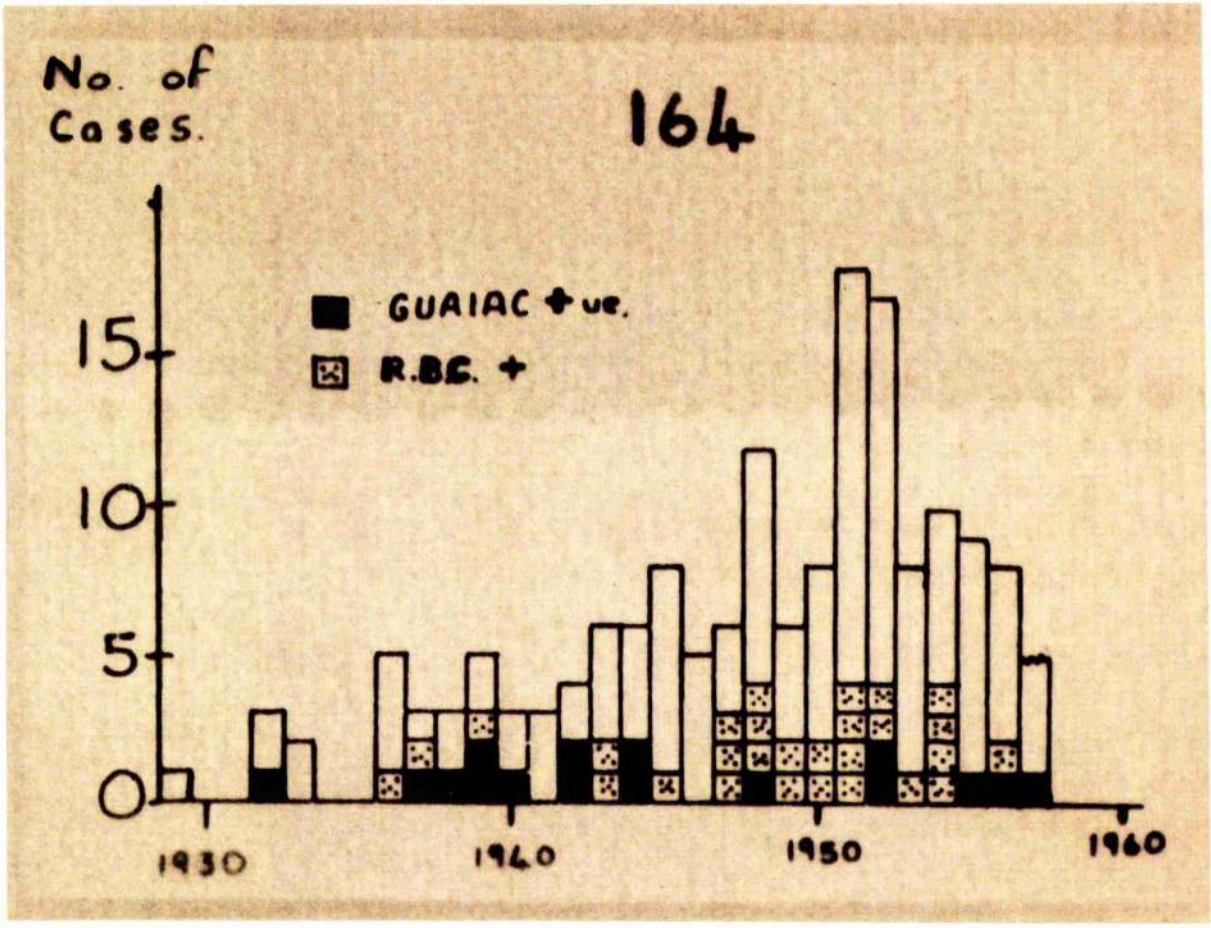
The Incidence of Nephrosis

The number of cases beginning each year during the period 1929 to mid-1957 is shown in Figure XLI. Throughout this time the total population of the area from which the children were derived did not vary widely. During the period 1947-1952 a marked increase in the birth rate occurred partly related to the return of the armed forces from the second world war. This may partly explain the increased incidence from 1948-1955. Since 1952 a number of cases have probably drifted to other paediatric units now in the area. The total population of the area from which these cases are drawn amounts to 2 - 3 million, or 400,000 - 600,000 children. Taking a round

figure/

FIGURE XLI

The Annual Incidence of Nephrosis
1929-57



The black squares represent cases with macroscopic haematuria and the stippled squares cases with microscopic haematuria

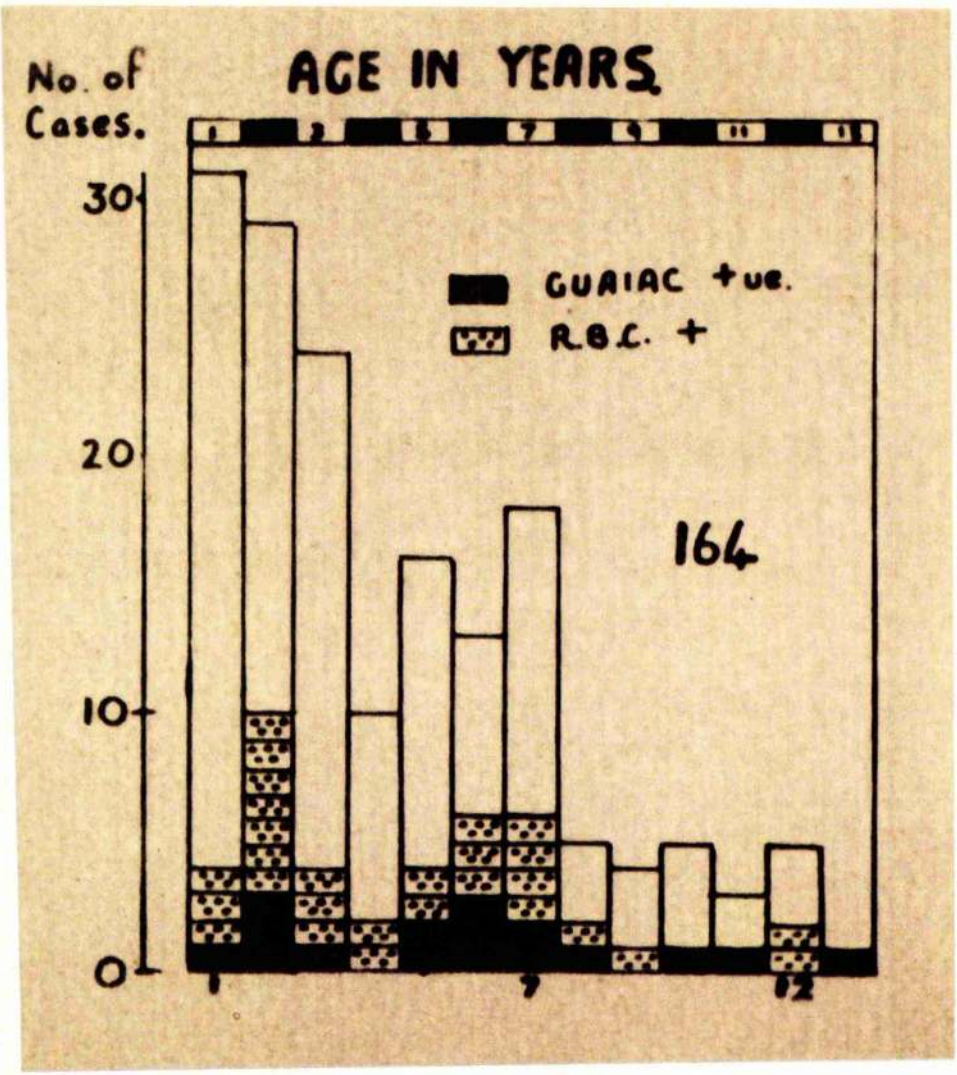
figure of 500,000 children aged 0 - 12 years in the area and the average admission of ten cases per annum (101 cases during the period 1948-57) the actual incidence of admission to this hospital is 2 per 100,000 children which may be compared with the figure of 2.3 per 100,000 for children aged 0 - 9 years in Cleveland, Ohio (Rothenberg and Heymann, 1957). There does not seem to be a seasonal incidence, since the greatest numbers of cases began in November, May and August and the fewest in December, March and October.

When the number of cases is related to the age at onset a very interesting picture unfolds (Figure XLII). Only four cases occurred during the first six months of life but the highest incidence is in the period from the seventh to the eighteenth month and no fewer than 48 cases (29%) fell into this twelve-month period. 21 per cent of the 168 children were aged less than one year at the onset of oedema as opposed to 2 per cent in the composite U.S. series of 425 cases (Barnett et al. 1952). A second significant rise in incidence was noted in children aged 5 - 7 years. Scottish children first attend school when aged 5 years and are there exposed to a variety of infections, bacterial and viral in origin. It is interesting to note a similar rise in incidence in this age group reflected in Todd's figures for Liverpool (Todd, 1957).

There were 98 boys and 66 girls in this group giving a male:female ratio of 3:2. This difference is not uniform throughout the various age groups. In the very young children (0-18 months old) there/

FIGURE XLII

Age at Onset of Nephrosis



Note the very high incidence during the first two years of life. In fact the peak lies in the period 7 - 12 months. Black areas represent cases with macroscopic haematuria on admission and stippled areas those with microscopic haematuria.

there is an almost equal incidence (25 males:26 females) but from the age of 18 months to 13 years there are 73 males to 40 females (1:8:1).

During the ten years 1948-57 the number of cases of nephrosis admitted to these wards was 101 and in the like period 328 children with acute haemorrhagic nephritis were admitted. The ratio of nephritis to nephrosis in this hospital is therefore 3.3:1. Since a child with acute nephritis is much more likely to be treated at home or in other hospitals than is one with nephrosis these figures are biased against nephritis.

History and Onset

The onset of periorbital oedema drew the attention of the parents to the illness of their child in 156 instances although the significance of this occurrence was frequently not appreciated until generalised oedema or ascites was noted in addition. In the remaining children swelling of the legs, or of the abdomen was the first sign to be noticed, and in a few occasions infections, usually pneumococcal in origin, drew attention to the illness. The period between the onset of recognised abnormality and admission to hospital varied from less than one day to more than one year. Twenty-eight per cent of cases were admitted within one week of onset, 78 per cent within one month of onset and 98 per cent within one year of onset. A number of the children had been treated previously in one or more of a variety of hospitals and earlier data concerning them were available.

The question of whether or not an infection, or a pyrexial incident had occurred prior to the onset of renal disease was raised in each case.

A/

A story of infection during the three weeks preceding oedema was obtained on 53 occasions (32%). The commonest infection was of the upper respiratory tract and was remarked on in 32 cases (20%). This infection was coryzal in type in 16 children and pyrexia with sore throat in 4 children. In the remaining 12 children the type of respiratory infection was indeterminate and presumably could be symptomatic of either viral or bacterial infection. This incidence of respiratory infection for the group could be explained as fortuitous if the average child in Glasgow suffered from three respiratory infections per annum which is not unlikely in an overcrowded city with the climate prevailing. In the group aged 7 - 18 months however respiratory infection closely preceded the onset of nephrosis in 23 per cent of cases and this relationship could only be explained by chance if on the average ten such infections occurred per annum. It should be emphasized that coryzal infection was most frequent and presumably was viral rather than bacterial in origin.

Overt infection was present in 33 children at the time of admission to hospital. In five of these peritonitis was present, three others had definite pneumococcal infections and twenty-five had a variety of other lesions including mild respiratory infections and a superficial form of cellulitis.

A history of immunising injection with nephrosis immediately following was given by six patients. Judged against the background of immunising procedures in young children this does not seem a significant incidence/

incidence but it cannot be denied that in individual cases the association of urticarial reaction to a first injection and nephrosis following a second seems difficult to gainsay. The suggestion of immunisation provoking nephrosis has been current for decades and it seems possible that it may contain an element of truth.

Sixteen (27%) of the children aged 2 years or less had ingested considerable quantities of mercurous chloride extending over a period of weeks or months prior to the onset of nephrosis. Six of the remainder had not received this chemical and 28 had ingested small amounts. Since ingestion of mercury was a very frequent practice in babies of the hospital class during the period 1940-1955 and because mercury was present in the great majority of such teething powders it is difficult to assess the significance of these observations. The total daily excretion of mercury was found to be significantly higher than in controls in several instances, but perhaps more significant is the fact that the incidence of nephrosis in children aged 6 - 18 months has dwindled rapidly since mercury was removed from teething powders in this area(1955-56). It is difficult to do other than incriminate calomel, but equally difficult to explain why the maximal incidence of such cases was during the period 1948-54 since calomel in considerable doses was prescribed for a number of infants by many medical practitioners at the beginning of the twentieth century. Teething powders are prescribed by grandmothers, chemists/

chemists and neighbours and given indiscriminately to a very large percentage of all infants, therefore it would seem likely that such cases represent an individual sensitivity to the drug rather than simple overdosage. Alternatively some other factor may have been operating during the period 1948-54. This might be the nephrotoxic effects of excessive calciferol widely used at this time.

Oedema

Periorbital oedema was noted at or prior to admission in 162 children (99 per cent), oedema of ankles or sacral area in 155 children (94 per cent), and ascites in 77 children (47 per cent.) Since ascites is indeterminate and difficult to detect in its early stages the last figure is largely subjective. There was no significant difference in the pattern of oedema in the children with and without haematuria. In some male children the scrotum was grossly distended and required support, in others the ascites caused the umbilicus to be everted and the heart and liver to be displaced by the increased intra-abdominal pressure. Gross periorbital oedema obstructing vision and increasing the liability to conjunctivitis was noted on occasions. Pleural transudates of varying amount were present in a few children.

The duration of oedema ranged from days, to weeks, months, years, or until the death of the patient. It was sometimes constantly and sometimes intermittently present, and static or variable in amount. The degree of ascites and of peripheral oedema did not necessarily run parallel and one may be present out of all proportion to the other.

In/

In the present series of 164 cases the duration of the oedematous state varied as shown in Table IV. Since deaths from infection had cut short the period of oedema in 39 children, the values are given relation to the remaining 125 children in whom diuresis actually occurred.

TABLE IV

Duration of Oedema	No. of Cases	
<3 months	51	41%
3–12 months	37	31%
13–24 months	18	14%
<24 months	19	14%
	125	

The duration of oedema tends to be shortest when no haematuria is present, being of less than 3 months duration in 45 per cent. of those with no haematuria, in 33 per cent. of those with microscopic haematuria and in 19 per cent. of those with macroscopic haematuria. The average duration of oedema is shorter in infants than in older children. Oedema lasted less than 3 months in 73 per cent. of children aged less than two years.

The degree of oedema fluctuates widely and wildly, presumably influenced by alterations in sodium and water retention. Partial diureses occur from time to time and complete shedding of oedema

may/

may occur spontaneously or follow treatment or acute infection. Such episodes of diuresis are accompanied by a dilution of the concentration of protein in the urine due to the increase in urinary volume. In the present group of children rapid diuresis with loss of oedema and ascites and a diminution of albuminuria occurred in 111 patients on 161 occasions. It is exceedingly difficult to decide if a diuresis has been produced by a pyrexial incident or some form of treatment. A child who is fevered perspires freely, and may vomit, and diarrhoea is a very common complication in young nephrotics. Water and sodium are lost by all these routes and oedema may decrease in consequence. It is only where clear cut increase in urinary volume occurs that diuresis has occurred. In the present series sustained febrile illness due to severe infection occurred in 74 children. Thirty-four (46%) of these children died and subsequent diuresis occurred in 21 (28%) of the 40 who survived. Oedema returned in 10 of these 21 patients in whom diuresis occurred and 4 of these children have since died.

It is often difficult to know whether or not treatment was responsible for producing diuresis. An oedematous nephrotic may be started on treatment one day and a diuresis begin the next day without any association necessarily existing between the two happenings. In general, the longer the duration of oedema, the more likely is diuresis beginning soon after the start of treatment to be related to the commencement of therapy. For instance, a boy had been oedematous continuously/

continuously for five years, prednisolone therapy was started and three weeks later he was free of oedema and has remained so for some years. This seems clearly to be cause and effect. In order that some idea of the relative importance of the various factors may be arrived at these diureses have been split arbitrarily into three groups:-

(a) diuresis within 10 days of an acute febrile illness

(b) diuresis within 20 days of commencing treatment

(c) diuresis in which neither (a) nor (b) or any other explanation is obvious

TABLE V

Factors Related to Diuresis

Time of Diuresis	All Causes	Post Infection	On Treatment	Unknown
Less than 3 months after onset	48	4	25	19
3-24 months after onset	57	14	26	17
More than 24 months after onset	13	3	8	2
Total	118*	21	59	38

(*Note that in seven children episodes of diuresis due to treatment and infection occurred at different times.)

The apparent frequency of improvement related to any form of treatment will be fallaciously high in the group where oedema persists less than 3 months/

3 months since at this time spontaneous remission is common. On the other hand diuresis following treatment will be more significant in those children in whom oedema had persisted for 24 months or more and steroid treatment has this property. Following diuresis one of three things may happen; either the oedema returns, or proteinuria continues without a return of oedema, or the patient becomes asymptomatic. It is interesting to compare the outcome of diuresis to the various known factors.

TABLE VI

Outcome of Remissions with Diuresis

	Number of Cases	RESULTING STATE		
		Dead	Proteinuric	Asymptomatic
Spontaneous	38	15%	7%	78%
Post-infection	21	19%	33%	48%
After treatment	59	15%	17%	68%
Total	118	16%	16%	68%

From these findings it is clear that optimal results arose from spontaneous remission but these were, of course, most common in the early stages of disease. Many of the remissions attributed to treatment were probably spontaneous but co-incident with the start of treatment. The prognosis in even the minority of children in whom diuresis occurred after/

after infection is worse than when diuresis was spontaneous or due to treatment.

Urinalysis

The urine of each child was tested for the presence of protein by acidulation and boiling, for the presence of haemoglobin by the Guaiac test and for red blood cells by microscopy. The volume in each twenty-four hour period was measured where practicable and an estimation of albumin content per litre made by the Esbach method, from which the total albumin loss per day was calculated. In a number of cases the daily urinary output of sodium, chloride and potassium was measured. Bidirectional partition chromatography was carried out on a number of urines, with particular emphasis being placed on the presence or absence of nephrosis peptide (Arneil and Wilson, 1953b).

Volume of Urine: The two outstanding features with regard to the volume of urine passed during the 24 hours were the oliguria during the period of oedema formation and the polyuria associated with diuresis. In the initial stages the daily urinary output might be as low as 200 ml. and during diuresis might rise to 4,000 ml. or more.

Proteinuria: Albumin accounts for almost the entire protein content of the urine of most nephrotics although small proportions of alpha, beta and gamma globulins may be present. For all practical purposes the terms 'proteinuria' and 'albuminuria' can be used as synonymous in nephrosis.

Proteinuria fluctuates greatly in duration and in both the amount/

amount of the total daily loss and the concentration per unit volume of urine in nephrosis. The amount present ranged from about 0.25 g./litre to 24 g./litre and from 0.25 g./day to 10 g./day. The proteinuria may be transient or may persist for a very long time, during which time the patient is asymptomatic. Amongst the 102 survivors of this series proteinuria had persisted in 14 children for 5 years or more and in 5 children for 10 years or more.

Where the course of the illness is fairly rapidly downhill proteinuria tends to remain gross and oedema to persist intermittently or continuously throughout life. In the majority of cases however (74%) the total daily loss of protein in the urine decreases after some weeks, months or years and the oedema gradually recedes. This decrease in proteinuria may lead to recovery, become a chronic state or be followed by relapse. Prior to 1951 proteinuria seldom recurred if it had once disappeared completely. (This should not be confused with a reduction in proteinuria). Steroid treatment has made this occurrence not infrequent and in fact transient loss of proteinuria followed by a return of this symptom is a common result of inadequately sustained steroid treatment.

Haematuria: The incidence of cases with microscopic or macroscopic haematuria at onset followed the annual incidence or incidence in relation to age fairly closely (Figs. XLI and XLII). This confirms the findings of Rothenberg and Heymann (1957). Macroscopic haematuria, as judged by a positive Guaiac test was present in 18 patients (11%), microscopic haematuria/

haematuria ($\doteq$ $>10^6$ R.B.C. in 12 hrs.) in 29 patients (18%) and 71% had no evidence of haematuria on admission. On a few occasions patients who had been free of haematuria at the outset developed this symptom some months after the onset of the disease. Heymann and Rothenberg (1958) showed that the red cells in the urine of these nephrotic urines are usually intact and the urine red in colour, on contrast to the many lysed cells and haemoglobin giving a smokey brown colour to the urine of acute haemorrhagic nephritis.

Electrolyte Content:

(a) Sodium During the period of oliguria and oedema formation the daily output of sodium was very low indeed, often amounting to less than 20 mg. per day. With rapid diuresis the level rose to as high as 6,000 mg. per day.

(b) Chloride These levels roughly paralleled those of sodium being as low as 30 mg. daily during oedema formation and as high as 11,000 mg. daily during diuresis.

(c) Potassium During the oedematous period and also during diuresis this value did not fluctuate so much as did those of the sodium and chloride. The potassium excretion did rise significantly after approximately one week of treatment with chlorothiazide and could lead to hypokalaemia if supplementary potassium were not added.

Findings in the Blood

The non-protein nitrogen (N.P.N.) content of the blood was determined in 161 patients at the time of their admission to hospital. This/

This value was less than 45 mg./100 ml. blood in 153 children (95%) and above this level in 8 cases (5%). The N.P.N. level was above 45 mg./100 ml. in 5 of the 18 children in whom macroscopic haematuria was present at the onset of the disease (28%) and in only three of the remaining 146 cases (2%).

The total serum protein level was estimated in 150 oedematous children soon after the onset of illness. The average value was 4.50g. of serum proteins per 100 ml. with a standard deviation (S.D.) of 0.77g. The proteins were fractionated chemically in 116 instances using the method described by Howe (1921). The values obtained were:

Average serum albumin level 1.85 g.100 ml. S.D. = 0.72 g.

Average serum globulin level 2.53 g.100 ml. S.D. = 0.47 g.

This represents an average so-called "albumin/globulin ratio" of 1.85/2.53. When the proteins were separated electrophoretically, using a modification of the methoddescribed by Cremer and Tiselius (1950) quite different values are obtained. This method was used to measure the serum protein fractions in 42 oedematous cases. The average level was found to be:

TABLE VII

Electrophoretic Fractionation of Serum Proteins

	g./100 ml.	Standard Deviation
Average total serum proteins	4.3	0.76
Average serum albumin level	0.9	0.38
Average serum α and β globulins level	2.8	0.84
Average serum γ globulins level	0.6	0.25

Whilst the very low serum albumin level is the outstanding feature the low average level of γ globulin is worthy of note since it may contribute to the high incidence of infection at this stage. In order to demonstrate the inaccuracy of the term albumin:globulin ratio the two methods of estimation were carried out synchronously on ten oedematous nephrotics. The results obtained were as follows:

TABLE VIII

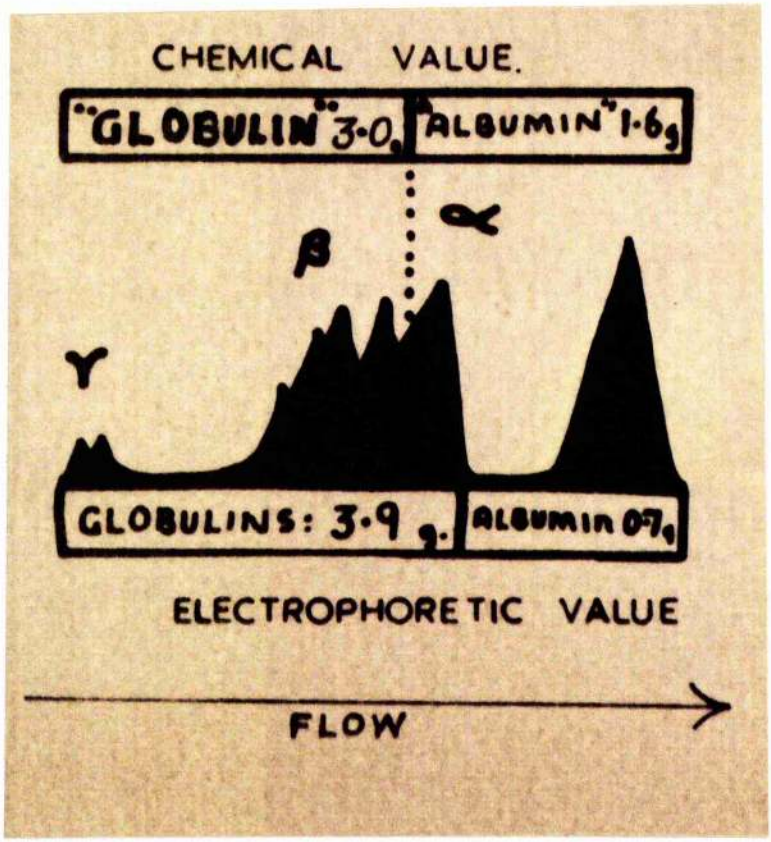
Average serum level	Chemical Method (Howe) g./100 ml. serum	Electrophoresis (Cremer & Tiselius) g./100 ml. serum
Albumin	1.6	0.7
Globulins	3.0	3.9
"Albumin/globulin ratio"	1:1.9	1:5.6

The explanation of this is that the chemical method separates albumin and some α globulins from the remaining globulins. These facts are illustrated in Figure XLIII.

A further interesting point arises from this discrepancy; when Rennie (1933) calculated the low levels of osmotic pressure obtaining in the serum of such cases the formulae of Govaerts' (1925, 1926) were used. These results depend on the quantities of albumin and globulin present. The merit of his observations has been widely recognised but Rennie's hypothesis is made stronger still if the electrophoretic values are accepted. The average osmotic pressure of the serum of these ten nephrotics/

FIGURE XLIII

Electrophoretic Pattern of Serum Proteins



Note the differing points at which the serum proteins are divided by electrophoretic and chemical methods.

nephrotics is 13 mm. of mercury when the chemical results are used but only 9 mm. if the electrophoretic results are employed. Whilst such quantitative figures based on electrophoretic techniques may not be absolute and their validity is controversial they are sufficiently accurate to demand further attention for this problem. The muddled thinking involved when using the older method is clearly revealed. What really matters is the low level of plasma albumin and not the relative proportions. There seems no reason why we should not say so in a simple fashion.

Throughout the period of oedema the levels of total serum proteins and of albumin tend to remain low. In general it is true to say that a nephrotic who has a serum albumin level of less than one gramme per 100 ml. will be oedematous whereas one with a serum albumin level exceeding one gramme per 100 ml. will be free of oedema. There are exceptions in both directions however and the best tests for oedema are still a rapid increase in weight or the presence of pitting on pressure or the presence of periorbital swelling!

Sooner or later the daily loss of protein in the urine decreases and the serum albumin level rises. The presence or absence of oedema does not always seem to vary directly with the level of serum albumin and the degree of secondary aldosteronism present is probably relevant. When proteinuria eventually settles to minimal amounts or disappears altogether the level of serum albumin returns to normal. In a series of 81 cases followed for 2 - 28 years, the following results were obtained.

TABLE IX

Electrophoresis of Serum Proteins 2 - 28 Years After Onset of Nephrosis

Status	No.	Serum Protein Levels g./100 ml.							
		Albumin		α & β Globulin		γ Globulin		Total	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Asymptomatic	60	3.7	0.6	2.2	0.5	1.4	0.5	7.2	0.6
Slight Proteinuria	9	3.6	0.8	2.4	0.5	1.4	0.3	7.4	0.8
Gross Proteinuria	12	1.8	1.2	2.7	0.7	0.9	0.4	5.4	1.1
All Cases	81	3.4	1.0	2.3	0.6	1.3	0.5	7.0	0.8

It will be noticed that the serum biochemistry of patients who were asymptomatic or had slight persistent proteinuria had returned to within normal levels. In such patients, when proteinuria remains gross, the total serum proteins, albumin, and gamma globulin are lower and the alpha and beta globulins are slightly elevated.

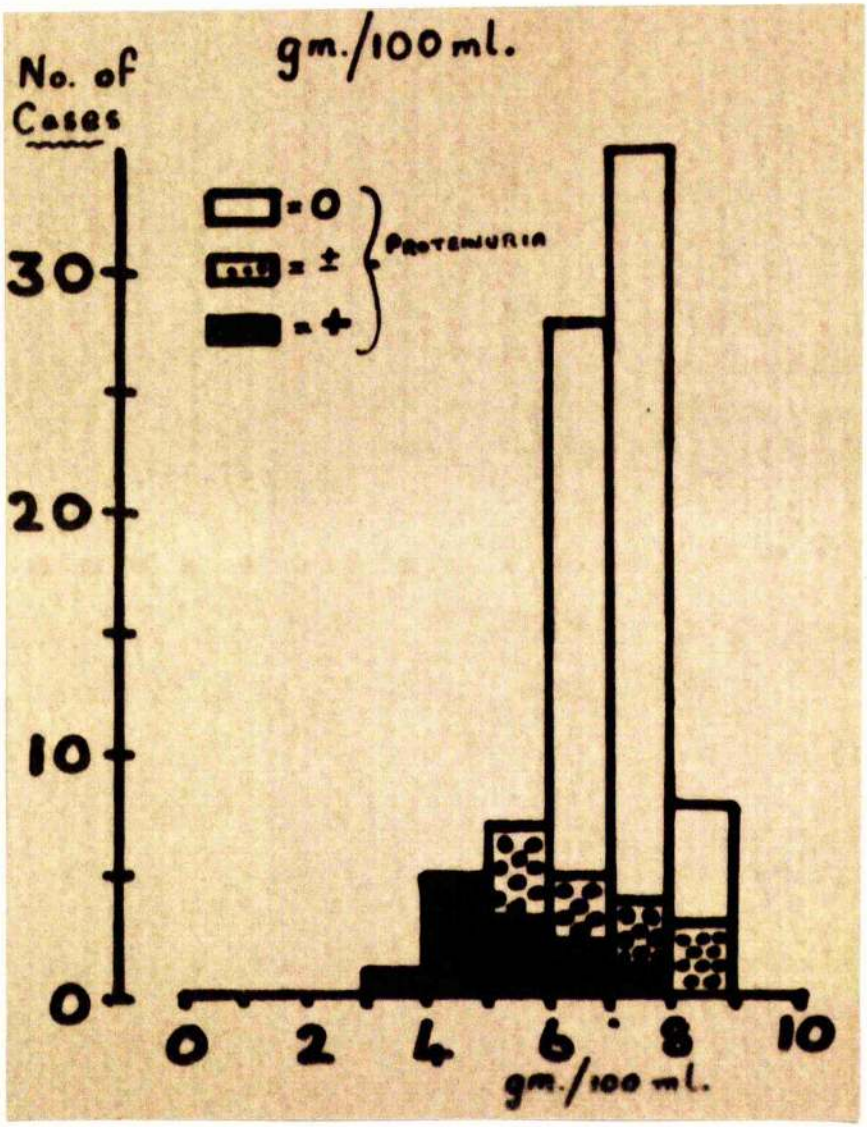
The relationship of these various fractions of serum biochemistry to the clinical status of the patient is shown in Figures XLIV, XLV, XLVI and XLVII. These clearly show the trends mentioned above.

Blood Cholesterol Level: The blood cholesterol level was estimated on 135 patients at the time of their admission by the method of Lieboff (1924). These results date from 1929 and the range of results is shown in Figure XLVIII. The mean value was 317 mg. per 100 ml. blood and the standard deviation 129. During this period (using chloroform extraction of whole blood) the range of average values

was/

FIGURE XLIV

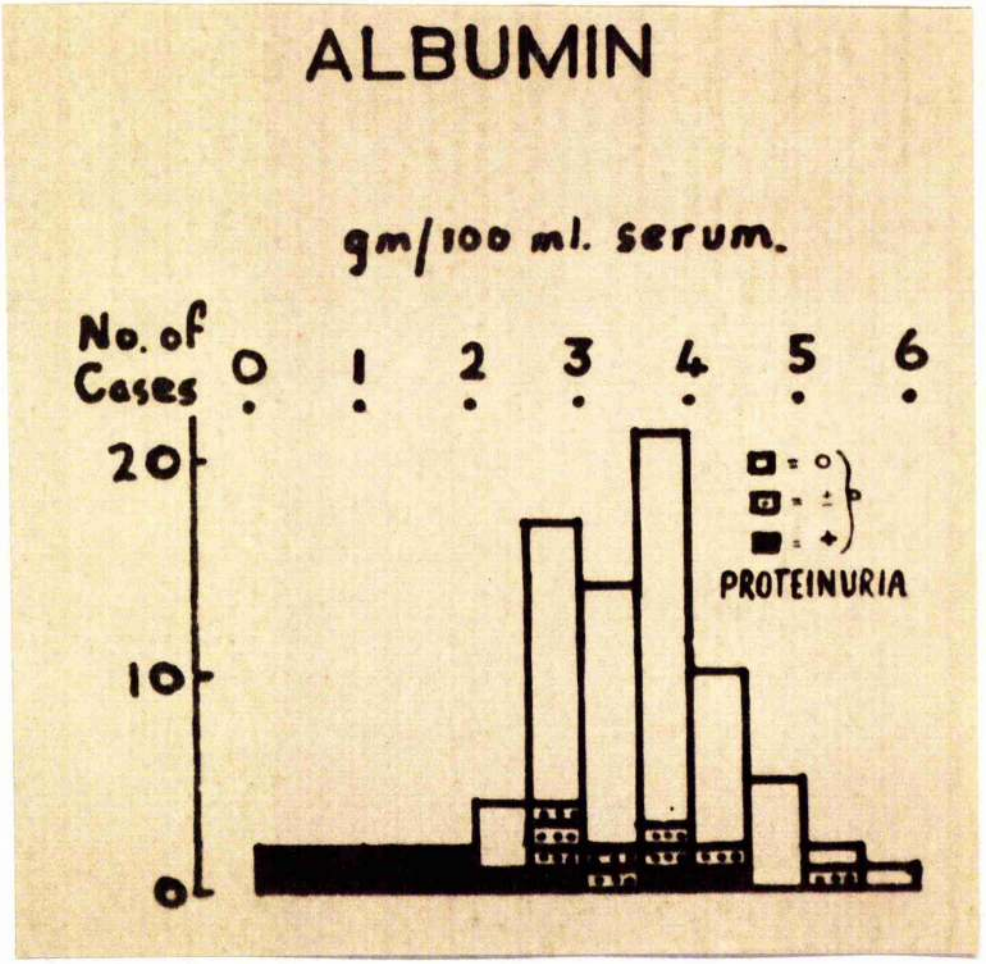
Total Serum Protein Levels in Nephrosis (2-28 years from onset).



The black areas refer to patients in whom gross proteinuria persisted and the stippled areas to those in whom slight proteinuria was present. It will be seen that the cases with proteinuria tended to have lower levels of total serum proteins.

FIGURE XLV

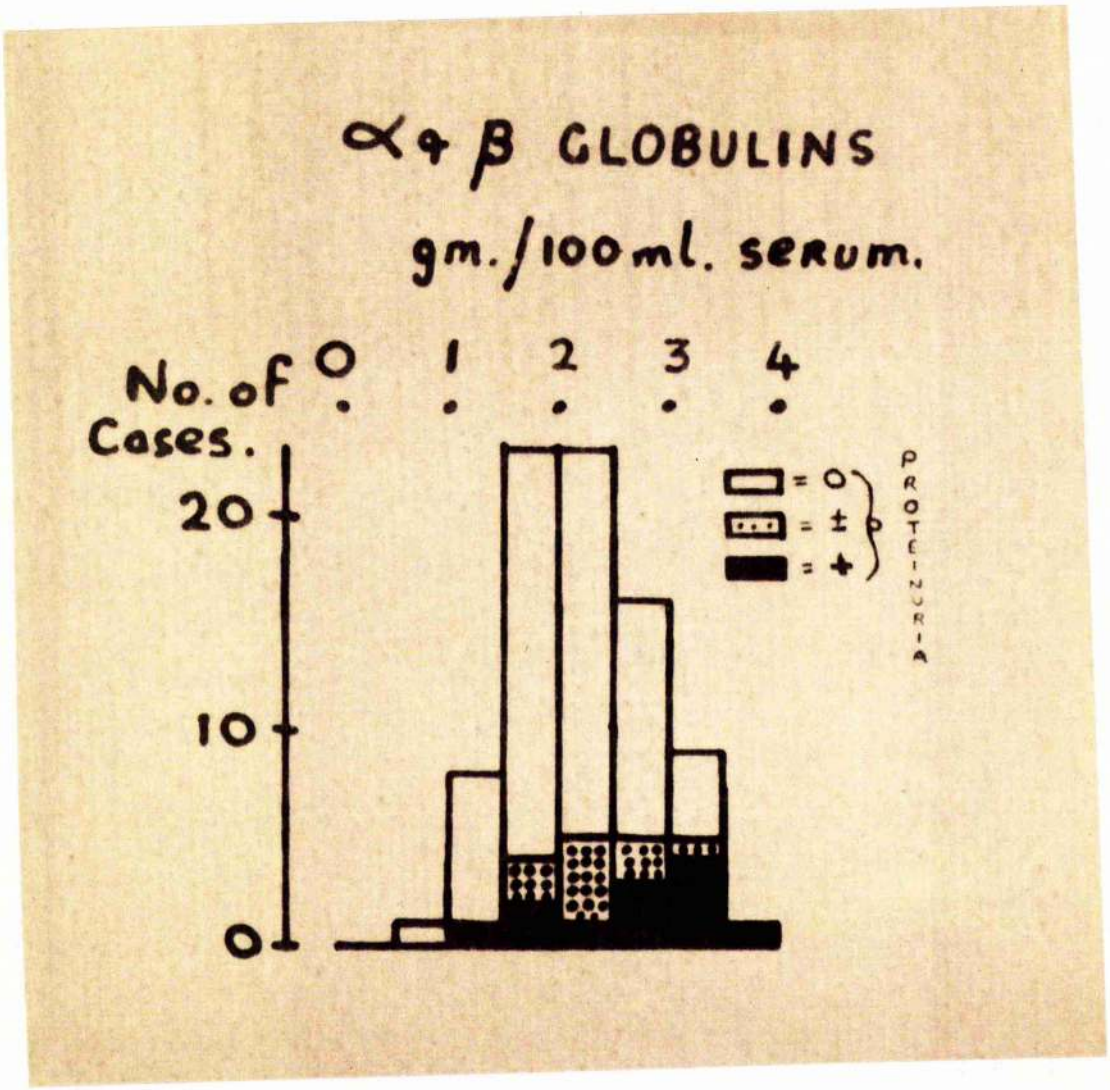
Serum Albumin Levels in Nephrosis (2 - 28 years from onset).



The black areas refer to patients with gross proteinuria and the stippled areas to patients with slight proteinuria. Note that the cases with proteinuria tend to have much lower levels of serum albumin.

FIGURE XLVI

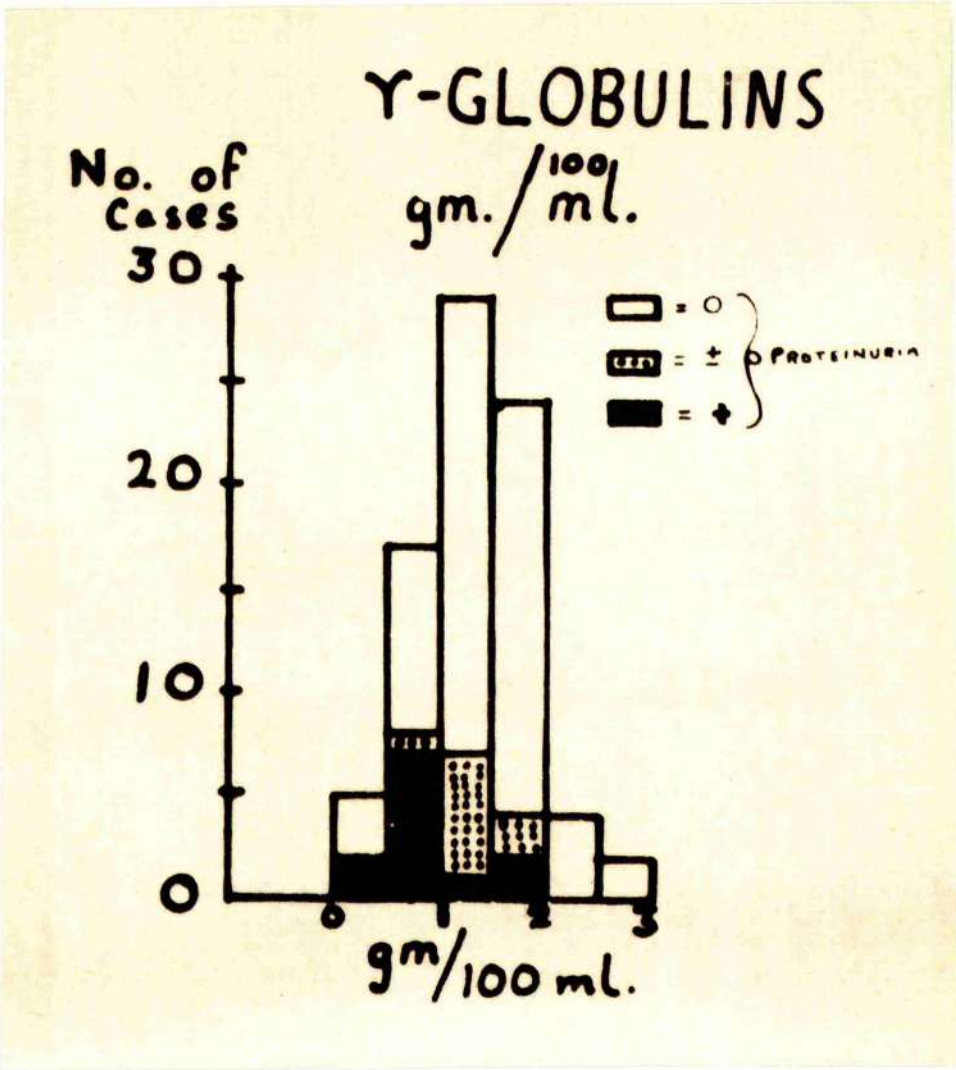
The Serum α and β Globulin Levels in Nephrosis (2-28 years from onset).



The black areas refer to patients with persisting gross proteinuria and the stippled areas to those with slight proteinuria. The levels of α and β globulin are higher in those with proteinuria.

FIGURE XLVII

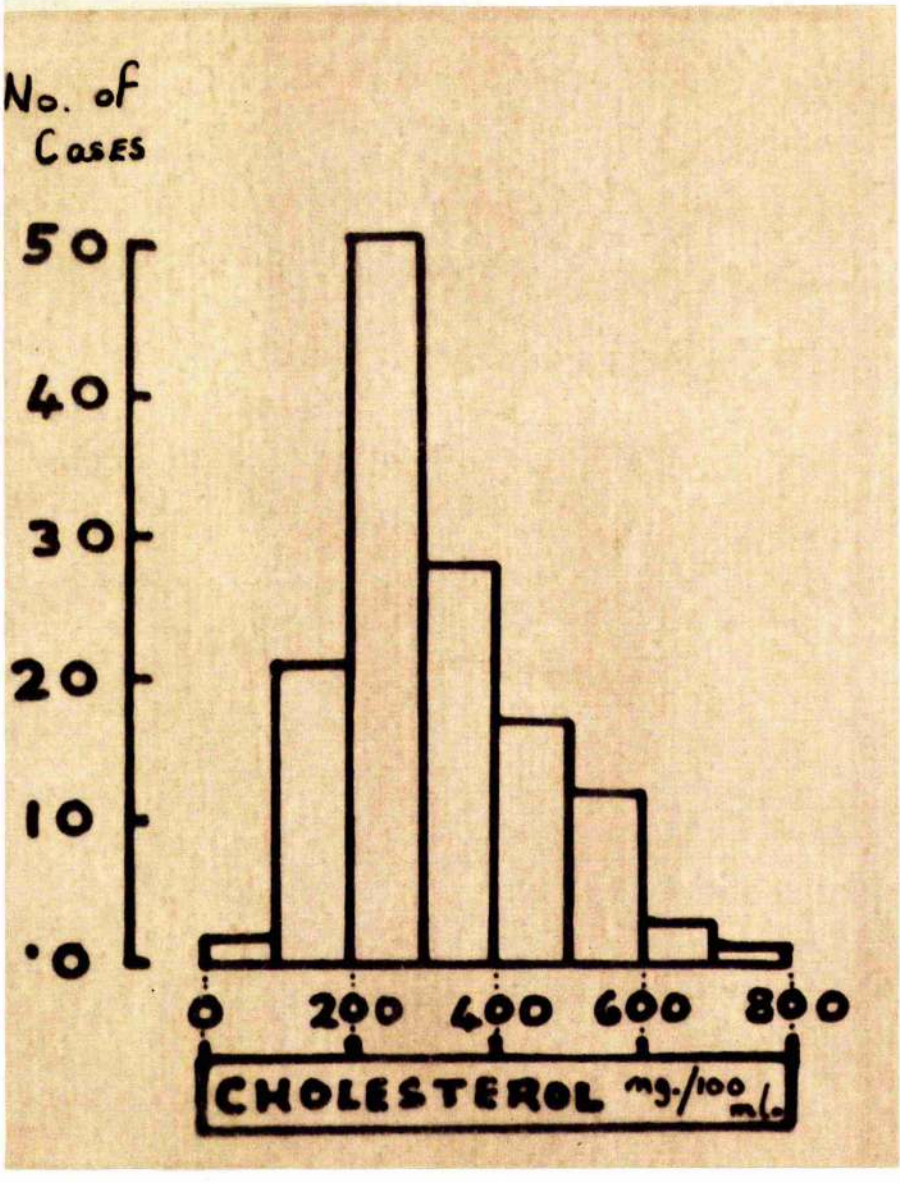
Serum γ Globulin Levels in Nephrosis (2-28 years from onset).



The black areas refer to patients in whom gross proteinuria persists and the stippled areas to those with slight proteinuria. The levels of γ globulin tend to be lower in those with proteinuria.

FIGURE XLVIII

Blood Cholesterol Level at Onset of Nephrosis



The method used was chloroform extraction of whole blood.

was accepted as 80 - 130 mg./100 ml. This scale of values is much lower than those obtaining when total cholesterol in serum is estimated by methods such as those of Zlatkis (1953).

The persistence of hypercholesterolaemia is a fairly delicate measure of continuing disease in the nephrotic subject. In general it may be said that when gross proteinuria persists the blood cholesterol will remain elevated but that a trace of proteinuria may remain without alteration of serum cholesterol values. The results of carrying out serum cholesterol estimations on 52 cases of nephrosis 2 - 28 years after onset were as follows:

TABLE X

	No.	Serum Cholesterol (mg.per 100 ml.)		
		Range	Mean	Standard Deviation
Asymptomatic Cases	52	40-140	89	25
Proteinuria Slight	7	67-140	87	22
Proteinuria Gross	6	65-280	195	85
Total	65	40-280	97	35

The method employed was chloroform extraction of whole blood. It will be seen that the values obtained for patients with persisting gross proteinuria were considerably higher than those for asymptomatic patients or those with minimal proteinuria.

These/

These trends are clearly shown in Figure XLIX.

Erythrocyte Sedimentation Rate: The erythrocyte sedimentation rate (E.S.R.) was measured in 58 children. In every instance it was greatly elevated and exceeded 30 mm. in the first hour whether carried out by the Westergren or Wintrobe method. In most instances the reading was in fact very much higher than this arbitrary level. The sedimentation rate remained elevated during the active period of the disease and seemed to be related to the level of serum albumin as measured by the electrophoretic method. The long term follow-up of cases revealed that where patients were asymptomatic the E.S.R. was within normal values. When proteinuria persisted the E.S.R. varied and when oedema was present it was always elevated.

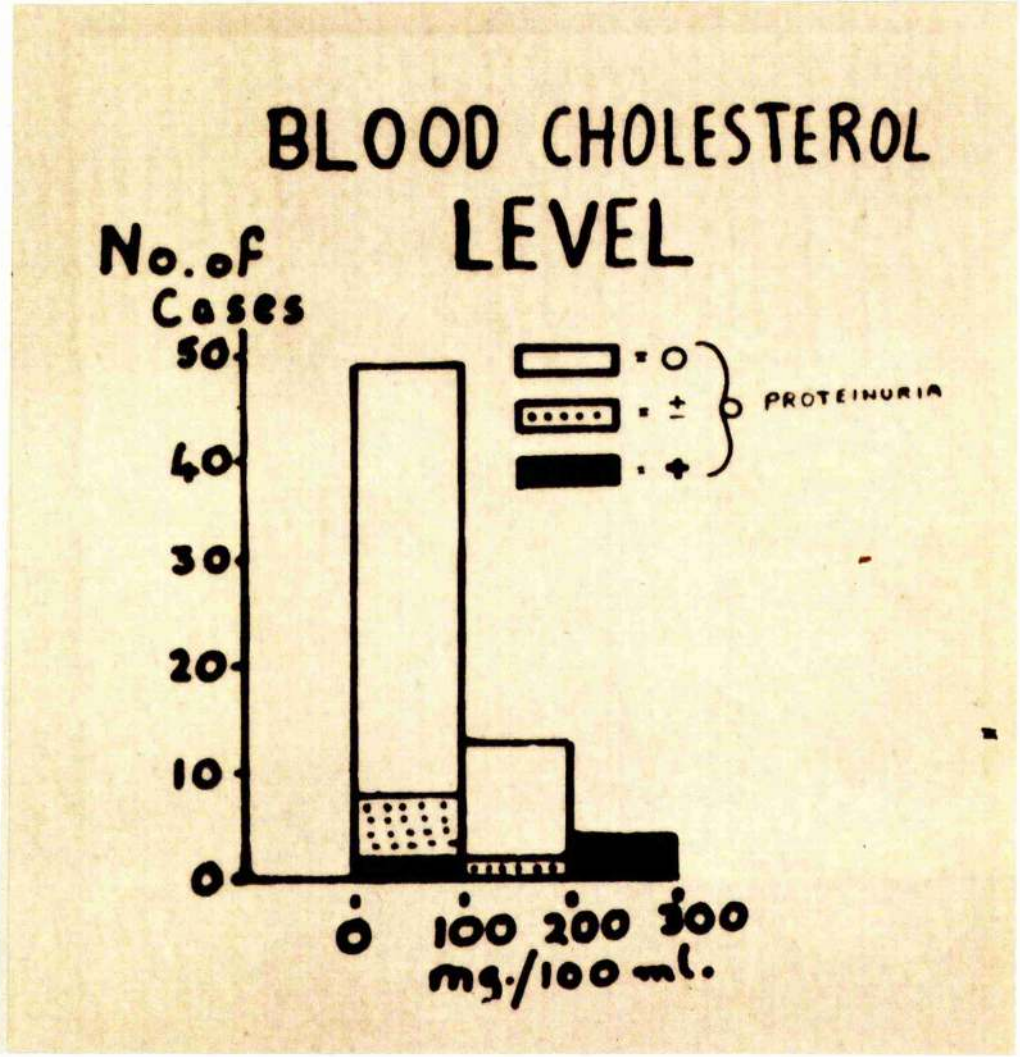
Blood Pressure: This estimation was carried out by sphygmomanometry and stethoscopic method at the time of admission. Definite hypertension was noted in 10 children. In 4 the hypertension was considerable and each of these had macroscopic haematuria. Slight elevation of the systolic blood pressure was present initially in 16 other children. Such minor elevations might have been emotional in origin or due to an experimental error occasioned by oedema of the subcutaneous tissues of the arm, or folding of the cuff.

In the late stages of the illness when renal failure begins to set in elevation of the blood pressure is not uncommon.

It seems therefore that apart from the late stages of the illness hypertension is uncommon in the nephrotic syndrome of childhood but is sometimes accompanied by other signs of glomerular damage such as/

FIGURE XLIX

Blood Cholesterol Levels in Nephrosis (2-28 years after onset).



The method used was chloroform extraction of whole blood. Those patients with persisting gross proteinuria (black) tended to have higher levels than those with no proteinuria or slight proteinuria (stippled).

as haematuria and nitrogen retention. In early nephrosis without haematuria the occurrence of hypertension is rare.

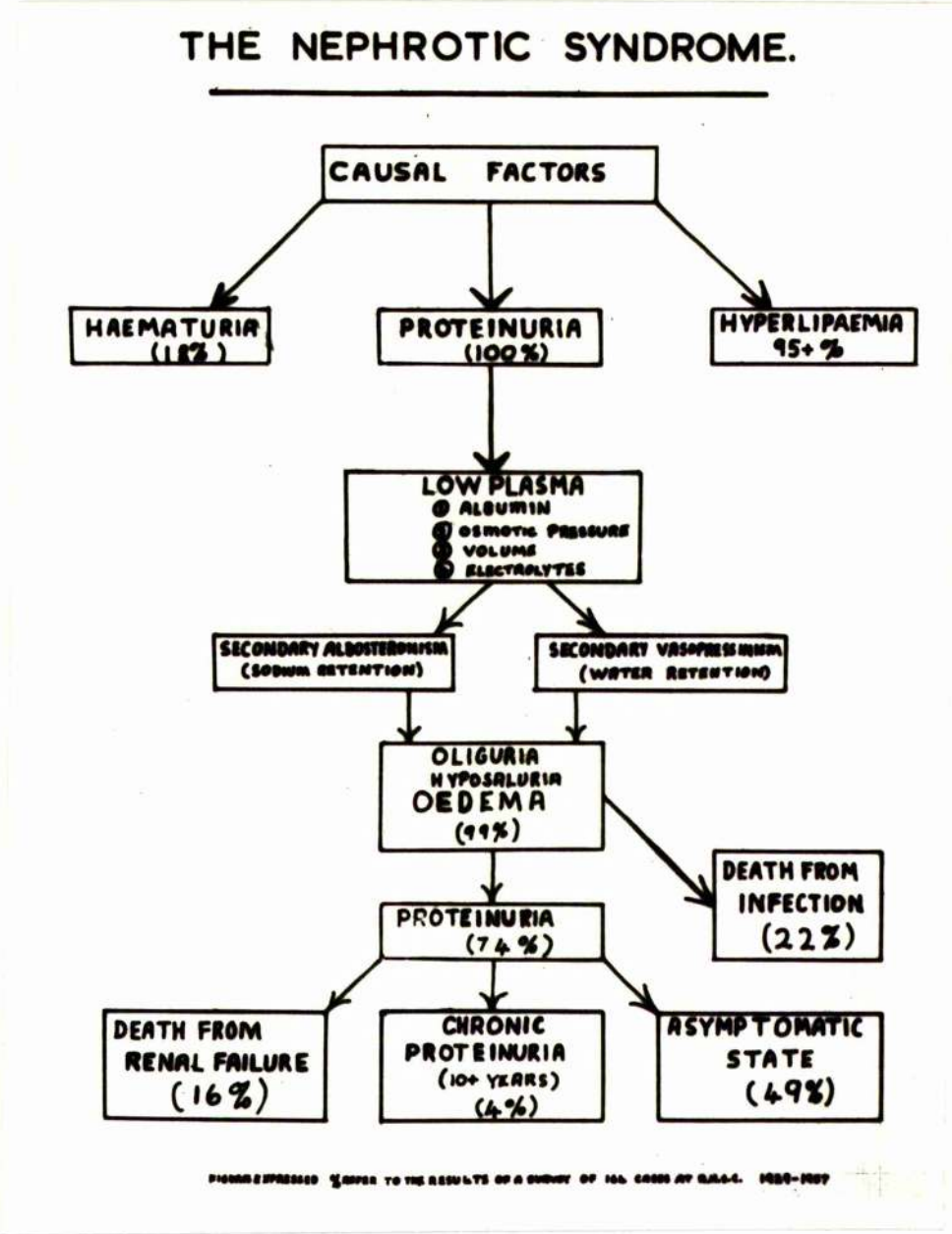
THE OVERALL PATTERN OF NEPHROSIS

The gross proteinuria of nephrosis is the basic and continuing process upon which innumerable variations are superimposed by the interaction of a variety of factors. Some of the influences relevant, such as the differing causal agencies, secondary aldosteronism and intercurrent infection, modify the natural tendency to recovery and their effects vary from producing rapid fluctuations in the degree of oedema present to causing sudden death. In one sense each case may be regarded as following a highly individualistic course but in practice cases fall into a pattern which is diagramatically shown in Figure L. This scheme is certainly an oversimplification of the problem, but serves to give one a background against which to assess the progress of a given case.

Until recently it had been thought that proteinuria did not satisfactorily account for the low plasma albumin level which sets in motion the train of reactions. One suggestion, that defective synthesis of albumin (either qualitative or quantitative in degree) might be responsible, has now been disproved. The findings of Gordon (1959) have raised the possibility that in the early stages of nephrosis albumin may be lost through the alimentary mucosa as well as through the nephrons and this hypothesis merits attention. The idea that the high plasma cholesterol/

FIGURE L

Schematic Representation of Nephrosis



(% figures refer to results of survey of 164 cases at Royal Hospital for Sick Children)

cholesterol might be due to loss of thyroid hormones by renal excretion is now discounted and it is seen as one facet of a general lipoprotein metabolic upset associated with proteinuria.

Our clinical impression was that many factors such as the age and sex of the patient, the duration of oedema and proteinuria and the presence or absence of haematuria were of considerably significance. Before dealing with these it is well to look at the various "typical" groups into which a large number of the patients with nephrosis readily divides (Fig. LI). It is against such a background of possible disease patterns (with many subdivisions) that the effect of therapy must be reviewed.

The Prognosis of Nephrosis

Prognosis will first be considered in relation to the entire group and then in respect of various subdivisions. The overall results for the 164 patients from 1929 - 1957 are as follows:

TABLE XI

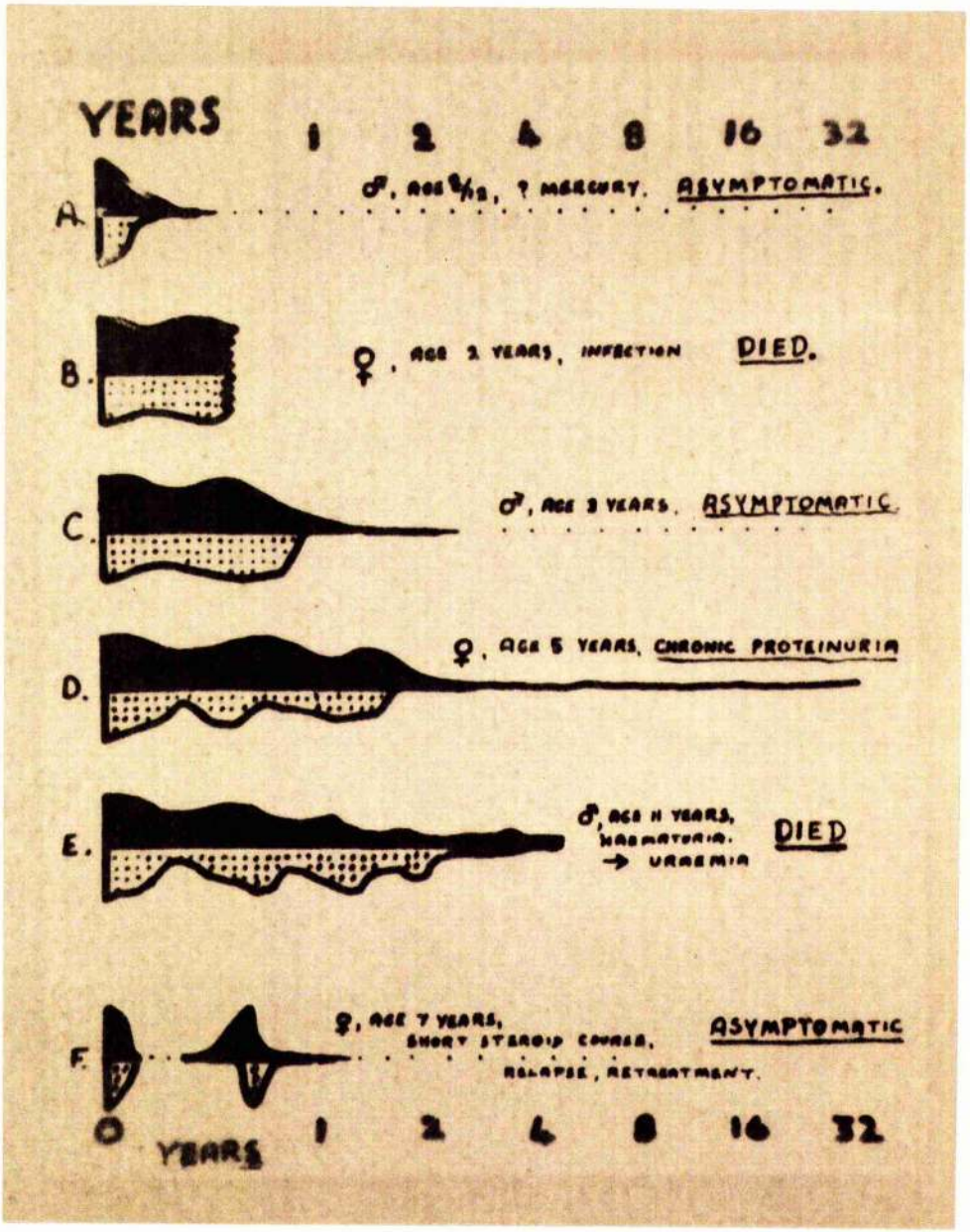
The Outcome of 164 Cases of Nephrosis

Status	Condition of patients			
	As at March 1959		Two years after onset	
	Number	%	Number	%
Asymptomatic	80	49	70	43
Proteinuric	22	13	50	30
Dead	62	38	44	27
Total	164		164	

It will be seen that there is a considerable difference between the status at/

FIGURE LI

Common Patterns of Nephrosis



Dark areas refer to proteinuria, stippled areas to oedema.

at two years from onset and some time later, due to the modification of the state of many patients whose proteinuria persisted for longer than two years. A long-term follow-up is always bedevilled by the inability to assess accurately the point in time when certain alterations in the clinical state occurred. The exception to this rule is that absolute occurrence, death. In the present series the date of death has been established for each child by scrutiny of the death certificate and/or post-mortem report. These may reasonably be considered as very reliable in respect of the relevant dates. In Table XII the length of time from the recognition of disease until the time of death of the 62 children concerned is recorded. Death occurred within two years in no less than 44 of these cases (72% of all deaths).

TABLE XII

Years Followed	Number Followed	Died	%	Died of Infection	%	Died of Renal Failure	%
0 - 1	164	35	21.3	28	17.1	7	4.3
1 - 2	164	9	5.5	5	3.0	4	2.4
2 - 3	159	5	3.1	1	0.6	4	2.5
3 - 4	151	6	4.0	0	0	6	4.0
4 - 5	142	2	1.4	1	0.7	1	0.7
5 - 6	132	0	0	0	0	0	0
6 - 7	124	0	0	0	0	0	0
7 - 8	107	1	0.9	0	0	1	0.9
8 - 9	89	2	2.2	0	0	2	2.2
9 -10	81	1	1.2	0	0	1	1.2
Estimated total deaths in the 164 cases after 10 years			39.6%		21.4%		18.2%
10-28	75	1	1.3	0	0	1	1.3

The actual cause of death cannot be obtained with the same degree of certainty as the date of death. By perusing the death certificate[8] of these/

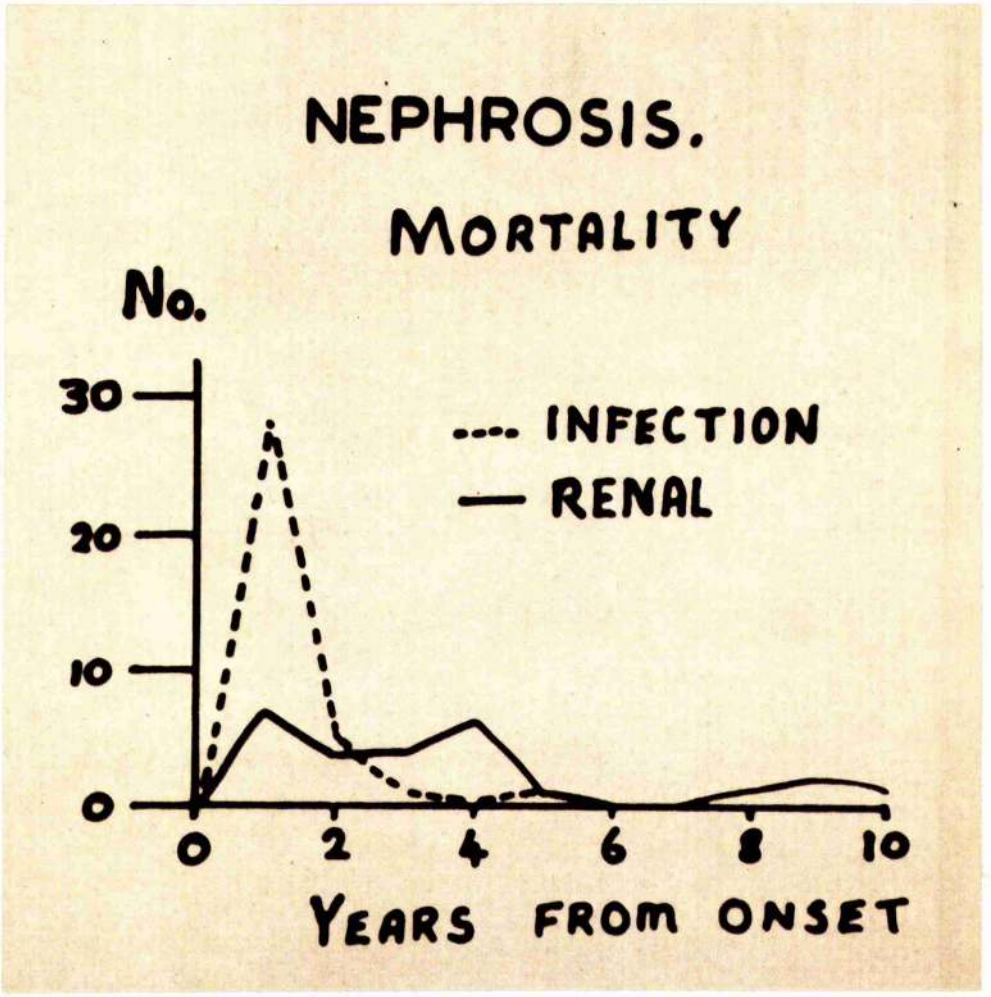
these children, interviewing the parents and practitioners concerned, and scrutinising hospital records and post-mortem reports however, a fairly comprehensive record has been obtained. If there is bias it seems likely that it lies in ascribing deaths occurring during the early years to renal failure, when in fact they were due to unrecognised respiratory or enteral infection occurring intercurrently in the grossly oedematous patients.

It was at once apparent that there were two principal causes of death. In the year following onset infection was by far the most significant factor but in later years renal failure became of importance. These facts are graphically displayed in Figure LII. It is also at once apparent from these figures that the mortality in any series of nephrotic patients can only be considered significant when related to the duration of the illness. In actual fact in this group of 164 patients, 75 were followed for periods of from 10 - 28 years and so far only one death has occurred after the tenth year of disease. Nevertheless the expectation of death in any series of cases must be weighted to allow for the number of years for which each patient has been followed and the method used to calculate the percentage mortality in the group allows for this (Table XII). Thus 62 patients dying out of a group of 164 gives a mortality of 37.8%, but if the figure is "corrected" to a percentage basis relating to length of follow-up it becomes 39.6% after ten years of illness. Thus, if the conditions prevailing persist one would anticipate that

$\frac{39.6}{100}$ /

FIGURE LII

Time of Death due to Infection and Renal Failure in 62 Nephrotic Patients



It will be seen that death from infection is practically limited to the first two years after onset.

$\left(\frac{39.6}{100} \times 164\right)$ children would be dead ten years after the onset of disease. This would be a total of 65.3 children or in other words 4 or 5 more of the remaining children should die between June 1959 and June 1967. Since these children have all survived at least two years these deaths should be due to renal failure (Fig. LII).

This method of calculation has been used to produce Figure LIII which is the calculated mortality for this group of 164 patients during the first ten years of illness. The deaths from infection and renal failure are shown separately.

Whilst it is comparatively simple to establish the date of death of these children it is very difficult to estimate when the proteinuria ceases. Accurate figures are available with regard to almost all cases up to the point two years from the onset of oedema. An attempt has been made to do likewise for cases followed for ten years or more and these results are given in Table XIII.

TABLE XIII

Status of Nephrotic Patients Two Years and Ten Years After Onset of Disease

All Cases	Point in Time	No.	Asymptomatic	Proteinuric	Dead
	2 years after onset	164	43%	30%	27%
	10 years after onset	81	56%	4%	40%
Less deaths from infection	2 years after onset	131	54%	38%	8%
	10 years after onset (approx.)	46	72%	5%	23%

These facts are rather difficult to grasp in tabular fom but diagramatically are quite lucid. Figure LIV illustrates the calculated progress/

FIGURE LIII

Calculated Percentage Mortality for Group During 10 Years Following Onset of Illness

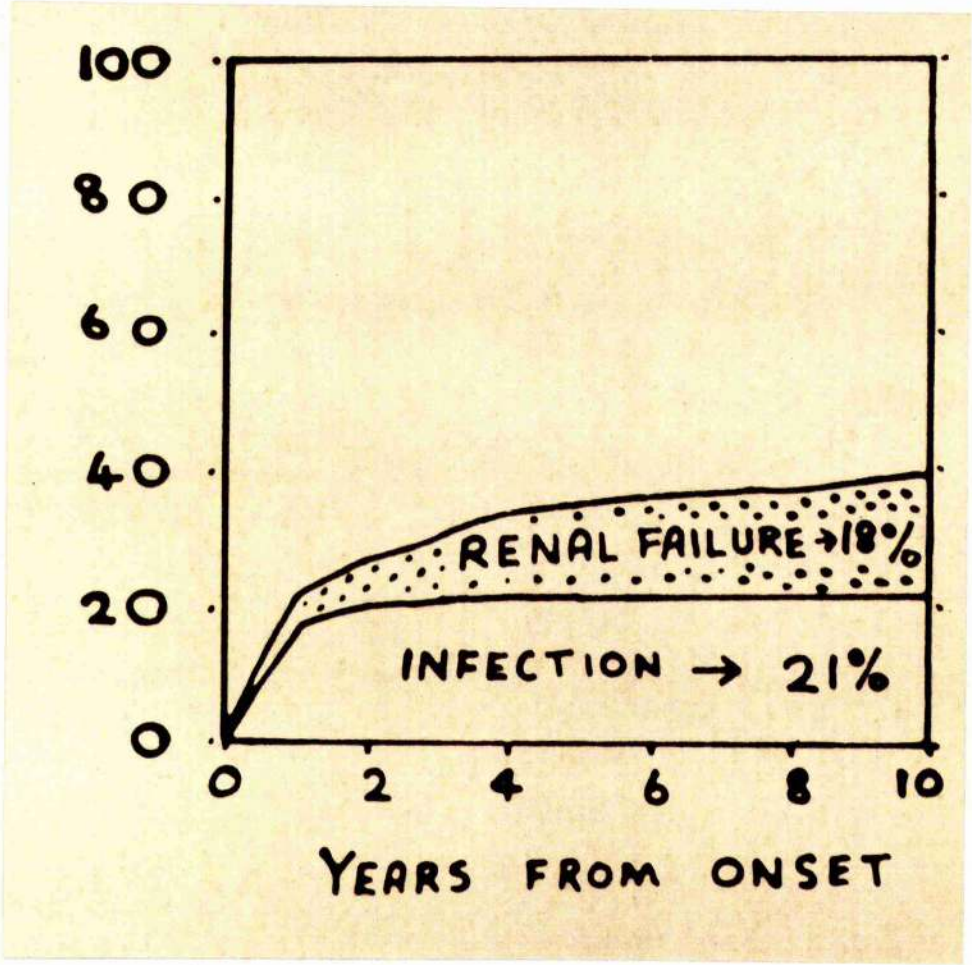
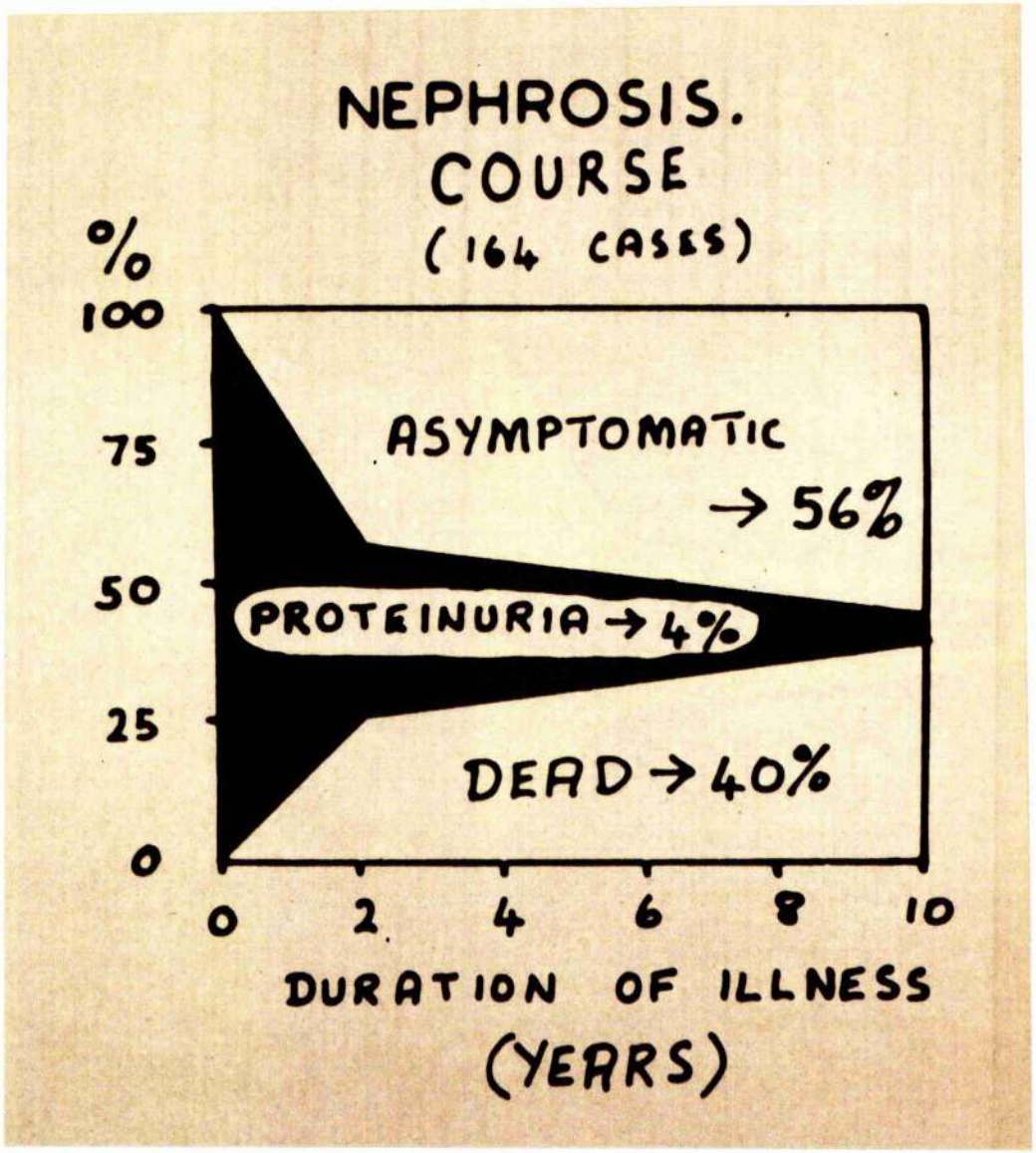


FIGURE LIV

The Course of Nephrosis During the First Decade Following Onset
(Calculated)



The greatest changes have taken place during the first two years. Note that of those children with proteinuria persisting at 2 years roughly 50% become asymptomatic after 10 years.

progress of this entire group of 164 patients during the ten years following onset of illness (provided the behaviour of cases so far followed for more than two years but less than ten years runs true to form).

Since infection, the major cause of death in these cases, is likely to virtually disappear it is interesting to recalculate these figures less deaths from infection (Table XIII, Figure LV). It is a remarkable fact that when deaths due to infection are allowed for during the period 1929-57 the number of patients asymptomatic after ten years of disease was 72%. Since the calculation depends on an unprovable premise (that the subsequent course of patients who died of infection would have paralleled that of survivors) this figure can only be regarded as speculative.

FACTORS AFFECTING PROGNOSIS

The relationship of the following factors to the outcome of nephrosis will now be considered:

(a) The duration of oedema

(b) The duration of proteinuria

(c) The age of the patient

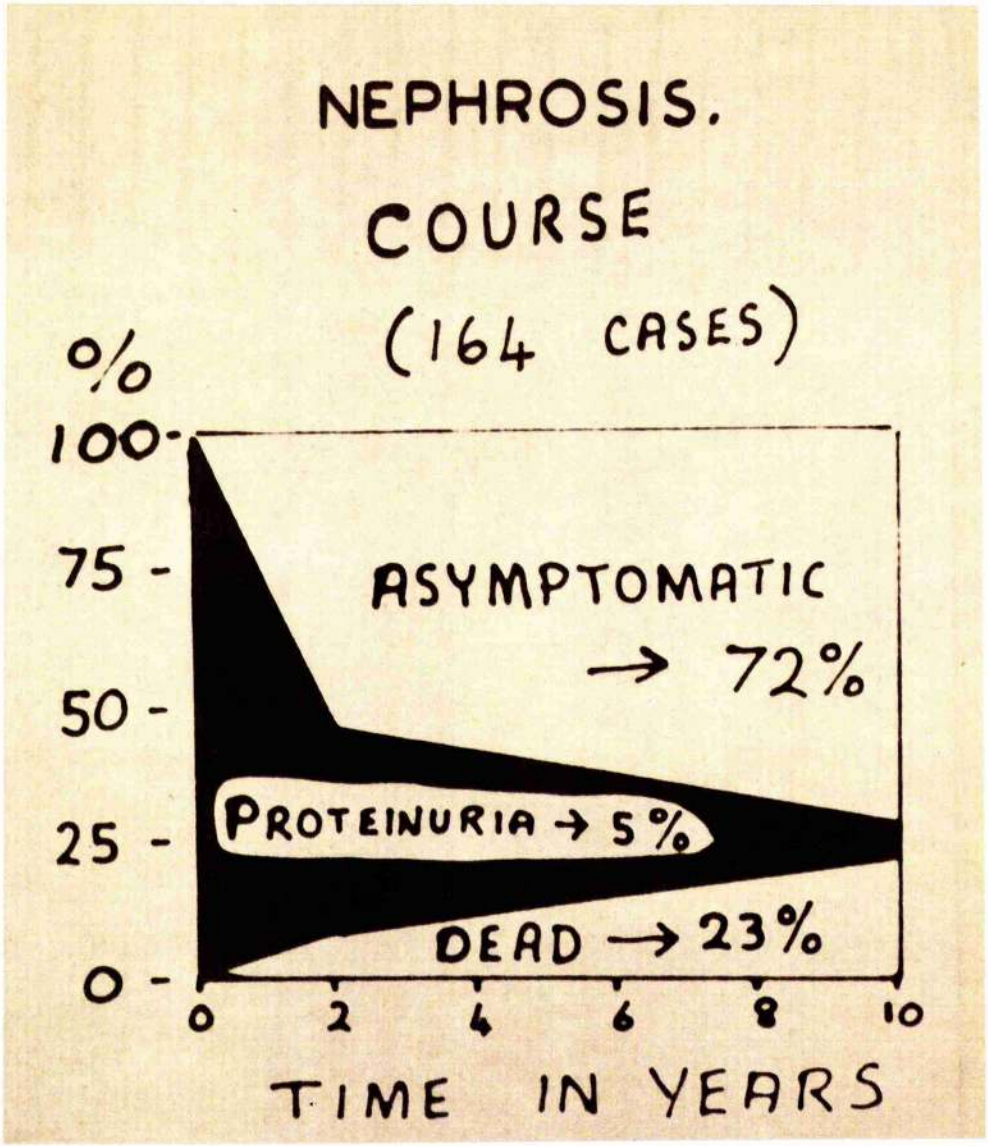
(d) The sex of the patient

(e) The presence of haematuria at the onset of disease

(a) The Duration of Oedema: The oedematous period is considered as commencing with the first appearance of oedema and terminating with the/

FIGURE LV

The Course of Nephrosis During the First Decade Following Onset
(Calculated less deaths from infection)



This represents the results which would have obtained if no deaths from infection had occurred. It also suggests the pattern of behaviour to be expected in the future if deaths from infection are eliminated and no improvement in renal function is effected by therapy.

the final disappearance of the sign. The presence or absence of peri-orbital oedema is usually readily perceptible and forms a relatively accurate index of onset. The persistent sacral or ankle oedema which is much less obvious to parent and unwary clinician is less accurately defined and may lead to error in underestimation of the period of oedema. Nonetheless the period may usually be assessed with reasonable accuracy. The overall results for the group are given in Table XIV.

TABLE XIV

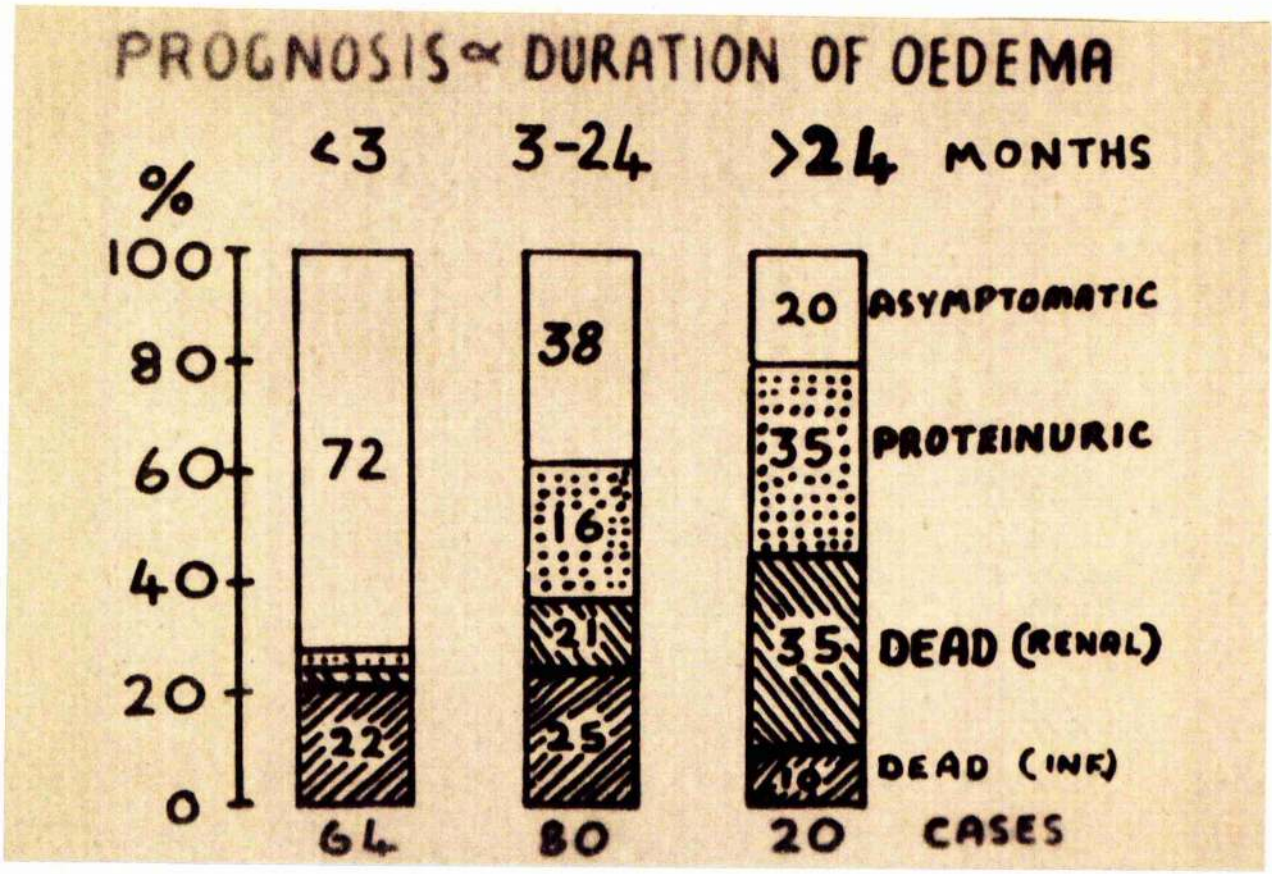
Duration of Oedema and Clinical Result in 164 Cases of Nephrosis

	Duration of oedema			
Outcome	Less than 3 mths.	3 - 24 months	More than 24 months	Total
Total number	64	80	20	164
Survivors	48 (75%)	43 (54%)	11 (55%)	102 (62%)
Asymptomatic	46 (72%)	30 (38%)	4 (20%)	80 (49%)
Proteinuric	2 (3%)	13 (16%)	7 (35%)	22 (13%)
Dead	16 (25%)	37 (46%)	9 (45%)	62 (38%)
Died of renal failure	2 (3%)	17 (21%)	7 (35%)	26 (16%)
Died of infection	14 (22%)	20 (25%)	2 (10%)	36 (22%)

These figures tend to be confusing and are probably more readily grasped in diagramatic form as in Figure LVI.

The interpretation of these results seems quite clear. The mortality rate and persistence of proteinuria are directly proportional to the duration of oedema whereas return to the asymptomatic state is inversely/

FIGURE LVI



The percentage of patients asymptomatic is directly proportional to the duration of oedema and the percentage dying or proteinuric is inversely so.

inversely related. It will be seen that many of the deaths (particularly during the first two years of oedema) are due to infection. Since these happenings may bias results and are probably no longer true these figures have been recalculated, excluding such deaths from infection (Table XV).

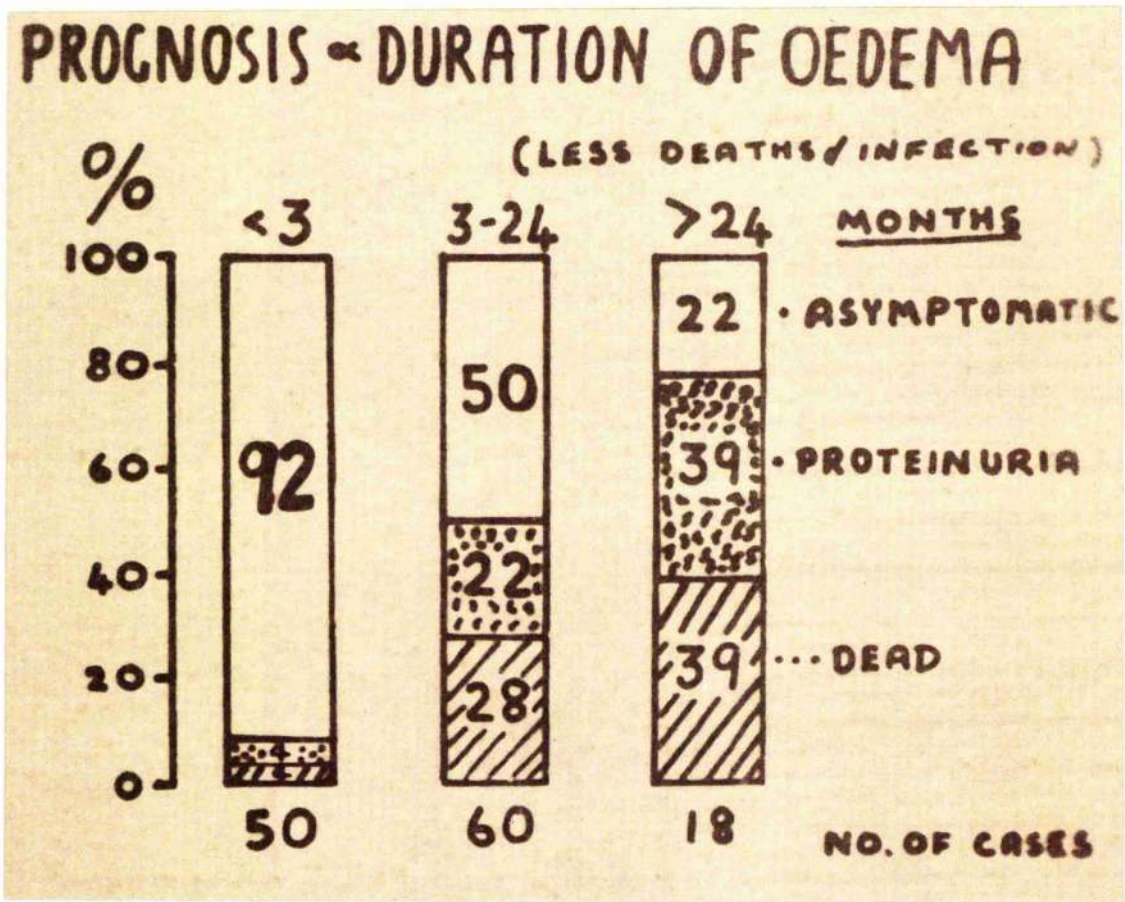
TABLE XV

Duration of Oedema and Clinical Result in Cases of Nephrosis (Less deaths from infection)

	Duration of Oedema			
Outcome	Less than 3 mths.	3 - 24 months	More than 24 months	Total
Total number	50	60	18	128
Survivors	48 (96%)	43 (72%)	11 (61%)	102 (80%)
Asymptomatic	46 (92%)	30 (50%)	4 (22%)	80 (63%)
Proteinuric	2 (4%)	13 (22%)	7 (39%)	22 (17%)
Died of renal failure	2 (4%)	17 (28%)	7 (39%)	26 (20%)

Once again these results have been graphically interpreted as shown on Figure LVII. This very clearly demonstrates once again that the eventual outcome may be directly related to the duration of oedema. The frequency of death, and of persistent proteinuria is directly proportional to the duration of the oedema, and the frequency of return to the asymptomatic state is inversely proportional. It should be remembered that no fewer than 92% of children in whom oedema persisted for less than 3 months are now asymptomatic and that only 22% of those children in whom oedema lasted two years or more are in this state.

FIGURE LVII



The percentage of children asymptomatic is directly proportional to the duration of oedema and the percentage dying or with proteinuria is inversely proportional thereto.

(b) The Duration of Proteinuria: Since the cessation of proteinuria is free of subjective sensation it stands to reason that patients are unable to state when such a change occurred. In very few instances did patients test their own urine regularly but the majority of children attended the Royal Hospital for Sick Children until proteinuria had ceased. In other instances it was only possible to say that the proteinuria had lasted for at least "x" months and less than "y" months. In other instances where proteinuria persisted in 1944, say six years after onset, and was still present in 1958, it was presumed that it had remained present constantly. For all these reasons the figures in this section must be regarded as approximations and less accurate than those relating to the duration of oedema. Table XVI contains these data concerning the entire group.

TABLE XVI

Duration of Proteinuria and Clinical Result in 164 Cases of Nephrosis

	Duration of proteinuria			
Status	Less than 3 mths.	3 - 24 months	More than 24 mths.	Total
Total No.	37	78	49	164
Survivors	24 (65%)	44 (56%)	34 (69%)	102 (62%)
Asymptomatic	24 (65%)	44 (56%)	12 (24%)	80 (49%)
Proteinuric	- -	- -	22 (45%)	22 (13%)
Dead	13 (35%)	34 (44%)	15 (31%)	62 (38%)
Died of infection	12 (32%)	20 (26 %)	4 (8%)	36 (22%)
Died of renal failure	1 (3%)	14 (18%)	11 (23%)	26 (16%)

These results are shown graphically in Figure LVIII. It will be seen that the proportion of patients becoming asymptomatic is inversely proportional to the duration of oedema. By the nature of the analysis no patients with proteinuria have had this sign for less than two years. The percentage of deaths is highest in the group of patients whose proteinuria lasted 3 - 24 months. Since a death by infection is likely to be the factor distorting results these results have been recalculated, discarding cases dying of infection (Table XVII).

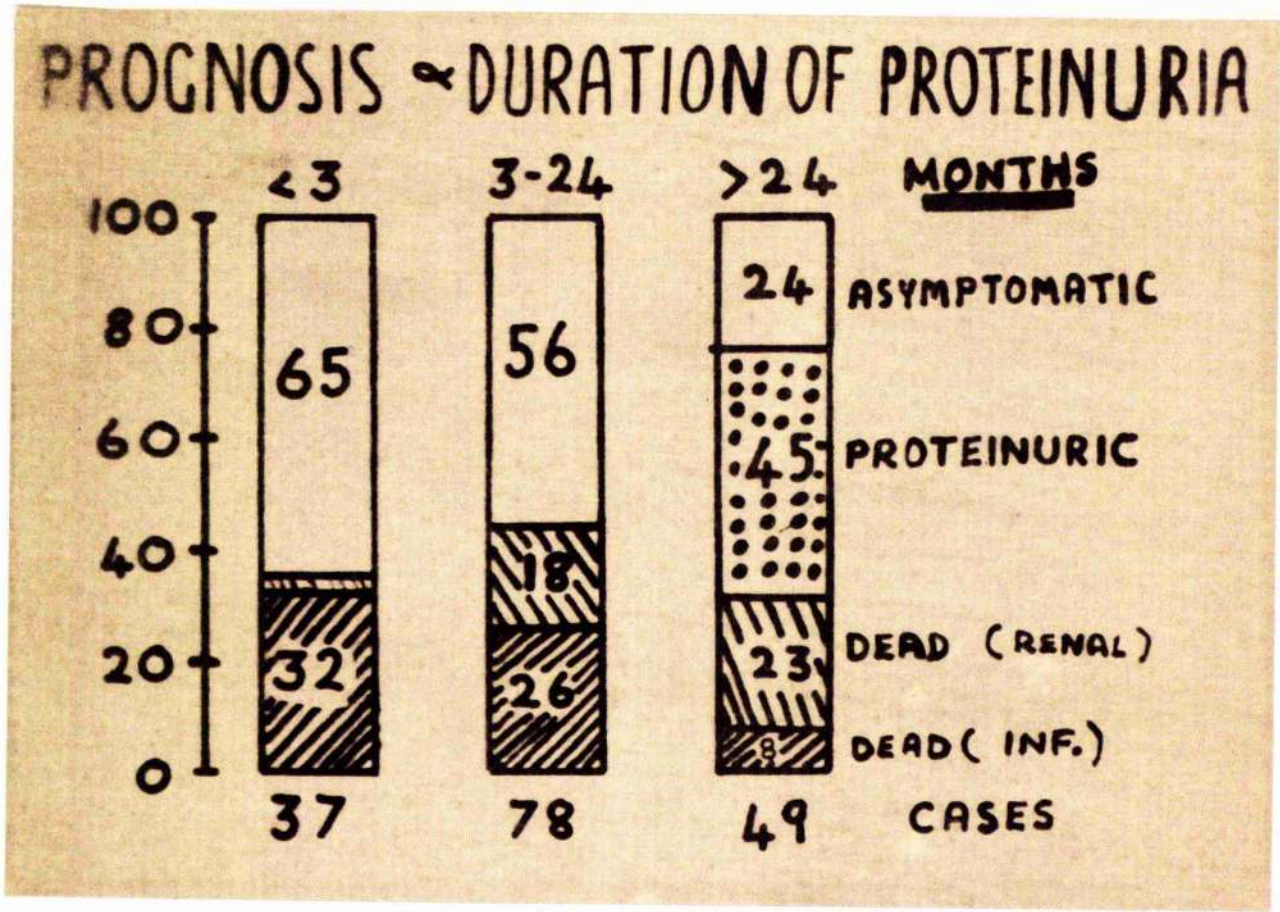
TABLE XVII

Duration of Proteinuria and Clinical Results in 164 Cases of Nephrosis (Less deaths from infection)

	Duration of proteinuria			
Status	Less than 3 months	3 - 24 months	More than 24 months	Total
Total no.	25	58	45	128
Survivors	24 (96%)	44 (76%)	34 (76%)	102 (80%)
Asymptomatic	24 (96%)	44 (76%)	12 (27%)	80 (63%)
Proteinuric	- -	- -	22 (49%)	22 (17%)
Died of renal failure	1 (4%)	14 (24%)	11 (24%)	26 (20%)

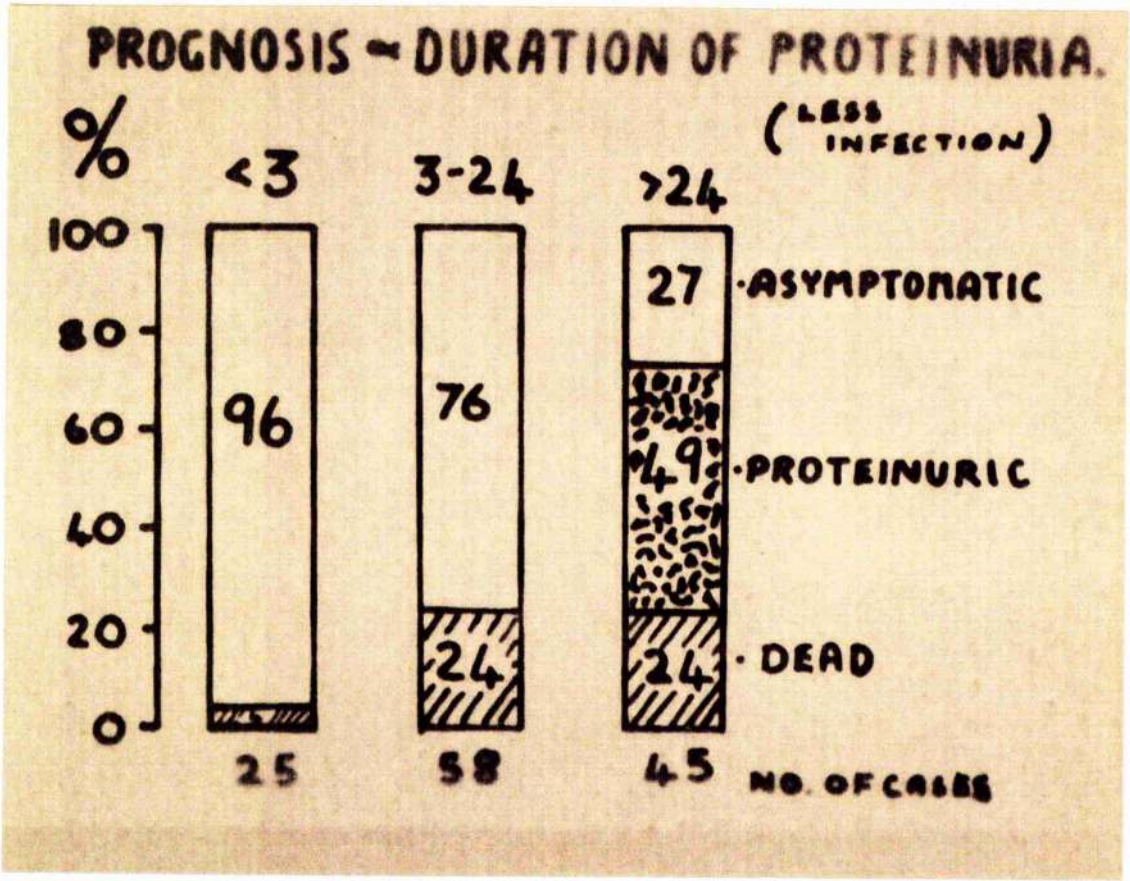
These results are shown graphically on Figure LIX which displays the direct relationship between duration of proteinuria and death or continuing disease and the indirect relationship of the duration of proteinuria and apparent recovery very clearly. It should be noted that where/

FIGURE LVIII



The proportion of children asymptomatic is directly proportional to the duration of proteinuria whereas the number dying is inversely proportional.

FIGURE LIX



The proportion of children asymptomatic is directly related to the duration of oedema and the number dying inversely so.

where proteinuria persisted for less than three months no less than 96% of patients became asymptomatic (apart from deaths due to infection). This is in marked contrast to the figure of 27% asymptomatic for children in whom proteinuria had persisted for 2 years or more.

(c) The Age of the Patient: A study of the distribution of age at onset of these 164 cases shows that there are two distinct components; (a) a very high peak between 6 and 18 months of age and (b) a steady incidence from 18 months of age onwards with a small peak at the age group 5 - 7 years. When these two groups are compared the findings are as shown in Table XVIII.

TABLE XVIII

A Comparison of Clinical Outcome in Children Aged (a) 1-18 months and (b) 1½-13 years at the Onset of Nephrosis

Clinical Status	Age at onset 1 - 18 months	Age at onset 1½ - 13 years
Number	51	113
Survivors	36 (71%)	66 (58%)
Asymptomatic	32 (63%)	48 (42%)
Proteinuric	4 (8%)	18 (16%)
Dead	15 (29%)	47 (42%)
Died of renal failure	3 (6%)	23 (21%)
Died of infection	12 (23%)	24 (21%)

It is clear that the death rate from renal failure at 21% is much higher in the older children as compared to the rate of 5.9% in/

in the younger group. This results in a much higher total mortality in the older children since death from infection was roughly comparable in each group (24% as compared to 21%). In addition the persistence of proteinuria is much more frequent in the older children (16%) than the younger ones (8%).

A visual comparison of these results is given in Figure LX. To confirm the validity of these results by allowing for the frequency distribution of duration of follow-up an estimation of mortality for the first ten years following onset has been made as was done for the whole group in Table XII. These results are seen in Table XIX.

TABLE XIX

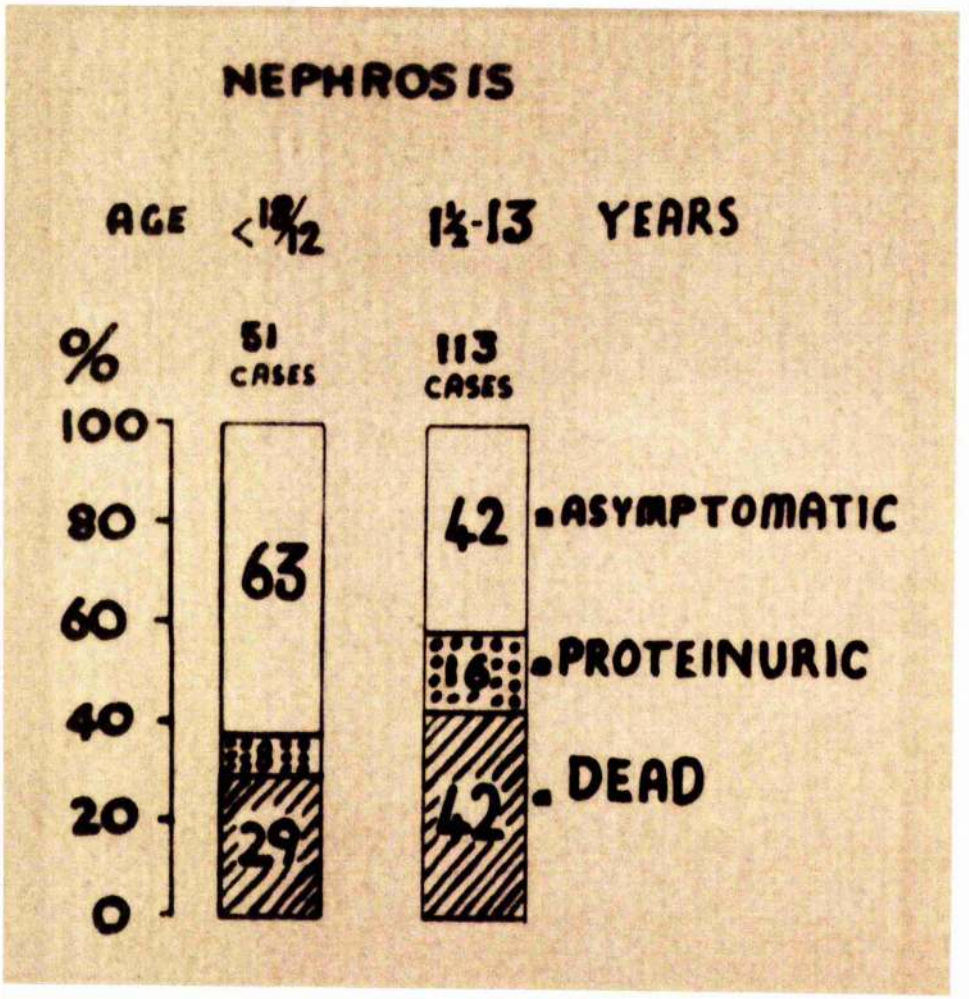
Calculated Mortality after Ten Years of Illness in Children aged 0 - 18 months and 1½ - 13 years at Onset of Disease

	Age at onset					
Duration Years	0 - 18 months No. followed	Died	%	1½ - 13 years No. followed	Died	%
0 - 1	51	13	25.4	113	23	20.1
1 - 2	51	0	0	113	9	8.0
2 - 3	51	0	0	108	4	3.7
3 - 4	50	0	0	101	4	4.0
4 - 5	48	0	0	94	3	3.1
5 - 6	46	0	0	86	0	0
6 - 7	43	1	2.3	81	0	0
7 - 8	40	1	2.5	67	0	0
8 - 9	34	0	0	55	2	3.6
9 -10	31	0	0	50	1	2.0
Total predicted			30.3			44.5

The calculated death rate is much lower in the young age group and a very much higher percentage of deaths in this group were/

FIGURE LX

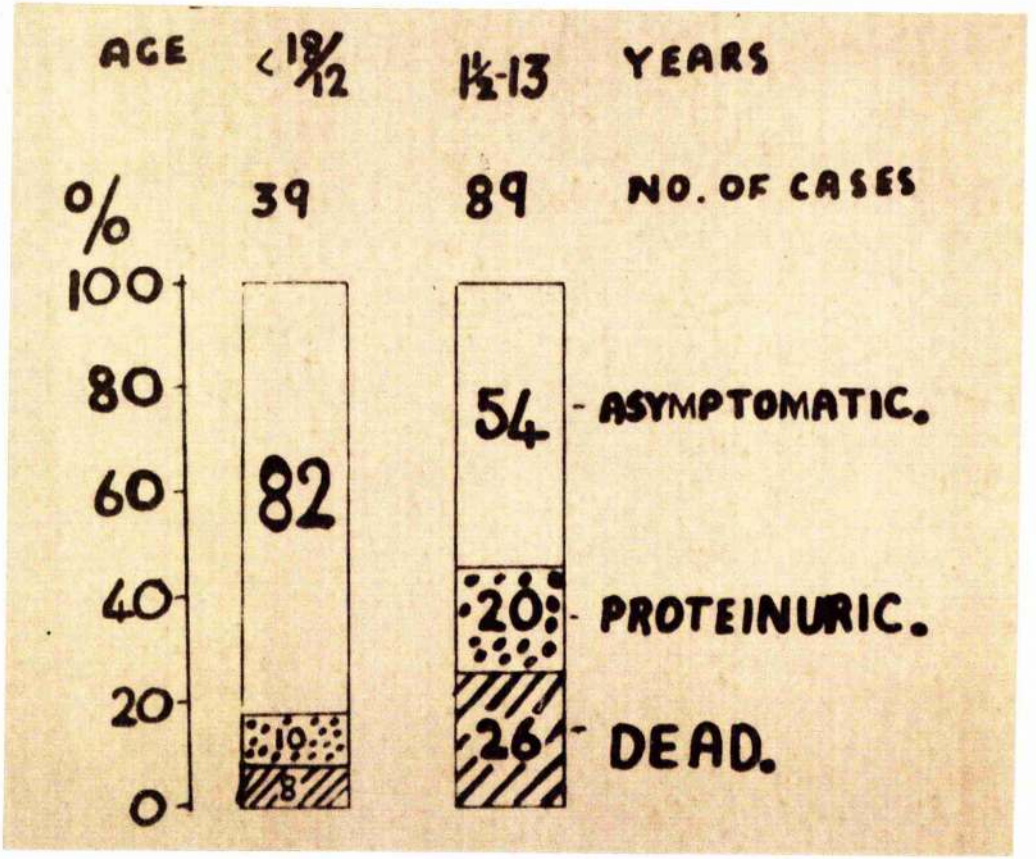
Comparison of Prognosis in Children aged 0 - 1½ years and 1½ - 13 Years at Onset



The mortality is lower and asymptomatic state more frequently achieved in the younger age group.

FIGURE LXI

Comparison of Prognosis in Children aged 0 – 1½ years and 1½ – 13 years at Onset of Nephrosis (less deaths from infection)



It will be seen that the prognosis for the younger group is much better when deaths from infection are eliminated.

were due to infection during the first year of illness. In young infants it is often possible to obtain a more clear story of incidents preceding illness than is possible in the more mature child whose life is much more complicated and who may already have suffered from other diseases. A careful enquiry into possible aetiological factors was made in each of the 51 cases aged 1 - 18 months at onset. In 12 children (23%) a coryzal infection had been noted during the 10 days prior to onset of oedema. This was occasionally accompanied by a few loose stools. It was not suggestive of streptococcal infection but seemed viral rather than bacterial in type. Glasgow is a grossly overcrowded and underprivileged city, and therefore the incidence of respiratory infection is high. Nevertheless in order to explain these findings by chance one would have to assume that acute coryzal infection occurred in each infant in the city on 10 occasions per year. This seems unlikely.

In 5 children (10%) reaction to immunising procedure seemed a possible contributory factor. These children, having reacted unfavourably to one immunising injection, developed nephrosis almost at once following a subsequent injection. This frequency of occurrence is not statistically significant but nonetheless is highly suggestive in individual cases. It may be that antigen-antibody reactions occasioned by infection or immunisation procedures aggravate rather than initiate proteinuria. Conversely it might be that latent hypogammaglobulinaemia increases liability to coryzal as well as pneumococcal infection.

In/

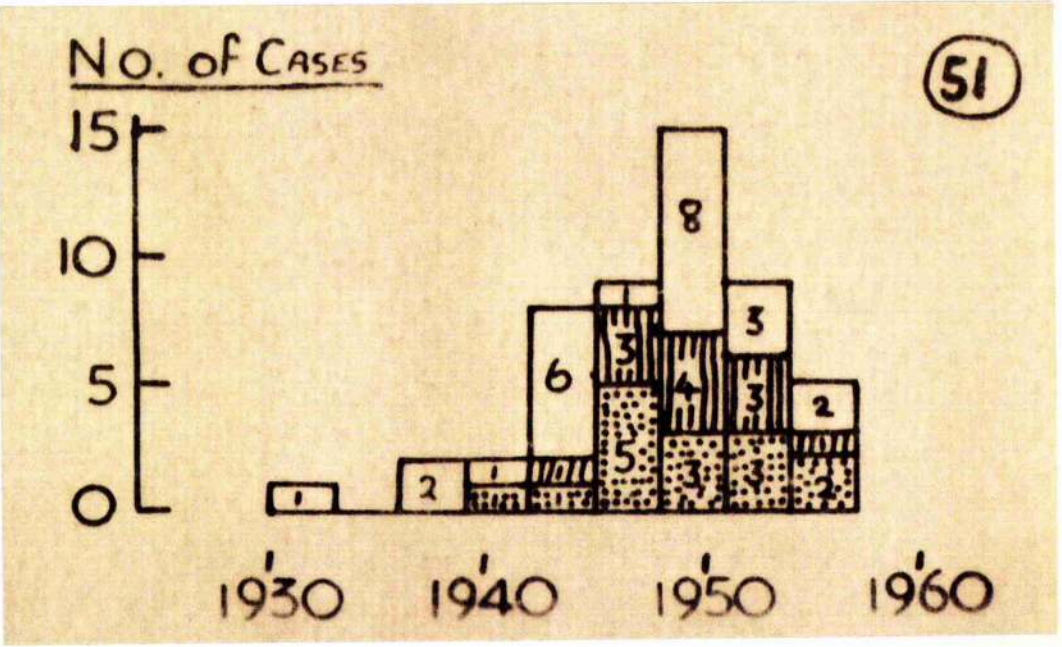
In 40% of children no contributory factors of any kind were obvious prior to the onset of oedema. Whilst this lack of information is in part due to bad history taking in the past, it is also possible that a considerable period of time had elapsed since the onset of proteinuria in these cases.

In 14 children (27%) a clear cut story of ingestion of mercurous chloride in quantities well above the average was obtained, and in several an excessive urinary output of mercury was measured. In Glasgow, prior to 1955, practically all babies of the hospital class received some calomel, usually at the behest of grandmothers, neighbours and chemists, on the pretext of soothing teething disorders. Since almost all babies were given some mercury it is difficult to incriminate this chemical directly, but it does seem highly suggestive that many of these babies developing nephrosis received larger amounts of calomel than usual. Since mercury was removed from most teething powders by 1955 one looked for a reduction in incidence. Figure LXII shows that in fact cases continued to occur during the period 1955-1957. If all such cases were due to mercury and no mercury had been dispensed to infants today one might expect the disease to disappear in this age group. Neither premise is absolute but a diminution in incidence has certainly occurred recently. As will be seen however, at least two of the five children in whom nephrosis arose in 1955-57 had received mercury prior to illness. During the period January 1958 to date no further cases have occurred in this age group.

(d)/

FIGURE LXII

Incidence of Nephrosis in Patients aged 1 year - 18 months
(51 cases)



This illustration shows that the number of cases occurring in this age group rose sharply between 1940 and 1950 and fell away thereafter. Since 1957 in fact there have been no further cases in this age group. The stippled areas refer to cases in whom a clear cut history of the ingestion of mercurous chloride in excess was obtained. The areas filled by vertical stripes refer to cases in whom upper respiratory infection immediately preceded the onset of oedema.

(d) The Sex of the Patient: It is somewhat surprising to note that the prognosis of the two sexes differs considerably. The results of analysis are shown on Table XX.

TABLE XX

Outcome of Nephrosis According to Sex of Patients

Sex	Male	Female
Number	99	65
Surviving	56 (57%)	46 (71%)
Asymptomatic	46 (47%)	34 (52%)
Proteinuric	10 (10%)	12 (19%)
Dead	43 (43%)	19 (29%)
Died of renal failure	18 (18%)	11 (17%)
Died of infection	25 (25%)	8 (12%)

A number of possible explanations might be advanced in an endeavour to explain these differences between the sexes. It might have been that a higher proportion of cases had occurred amongst females than males of recent years. This was not so since 48 of the last 76 cases were males, giving a ratio of males:females = 1.7:1 (cf. 1.5:1 for the entire group). Another possible explanation could have been that the male children were, on the whole, younger than the females. This again was not true since 42% of males were aged less than 1½ years at the time of onset of illness whereas 15% of females fell into this age group.

Another possible factor introducing bias could have been the presence/

presence of haematuria at the onset of disease. In fact however initial haematuria was present in 15 females (23%) and in only 14 males (14%). This should have biased the results in the opposite direction since nine of these fifteen girls with haematuria died subsequently. It seems that the difference is explicable on grounds of liability to infection since the deaths from renal failure amount approximately to the same proportion, whereas deaths from infection are commoner in males.

(e) The Presence of Haematuria at Onset of Oedema: The finding of haematuria of significant degree at the onset of nephrosis was recorded in 29 instances. A comparison of the course of these patients with that of the remaining 135 cases is of interest (Table XXI).

TABLE XXI

Comparison of Clinical State of Patients with and without Initial Haematuria

	Haematuria	No Haematuria
No. of cases	29	135
Survivors	11 (38%)	91 (67%)
Asymptomatic	10 (35%)	70 (51%)
Proteinuric	1 (3%)	21 (16%)
Dead	18 (62%)	44 (33%)
Died of infection	11 (38%)	25 (19%)
Died of renal failure	7 (24%)	19 (14%)

It therefore seems that the death rate in children with initial gross haematuria was 62% compared to 33% for the remaining children. It should be/

be emphasised that the onset of haematuria does not, as a rule, significantly precede the period of gross proteinuria and oedema but the onsets appear to virtually coincide. This initial gross haematuria may be accompanied by hypertension and/or nitrogen retention.

Since a few red blood cells are normally present in urine it should be clear that it is quite impossible to differentiate small increases in the presence of such cells unless a count is made of the total content of the urinary output for 24 hours. Since this was not done it is only possible to compare cases in whom gross haematuria (Guaiac test +ve or red blood cells increased on examining microscopically) with those cases in whom no red blood cells, or very few red blood cells were seen microscopically.

PART III

STEROID THERAPY OF NEPHROSIS

In 1951, when this investigation began, little was known of the actions of cortisone and ACTH, or of their effects on nephrosis. The methods of treatment employed during the period 1951-1959 have repeatedly altered in line with the increased understanding of the action of these hormones at the relevant time. Broadly speaking steroid therapy has passed through three main periods. During the first period (1951-54) short-term courses of cortisone and ACTH of 5 - 12 days duration were employed. During the second period (1955-57) progressively longer courses, of higher dosage, of more active compounds have been used. In the past two years new compounds such as triamcinolone, methylprednisolone, dexamethasone and chlorothiazide have become available and have been used. In 1951 the variability of the oedema in nephrosis remained as mysterious as the pathogenesis of the disease and even at a later date the most exhaustive investigation of Eder et al. (1954) emphasised the "unknown aetiological factors" in such anasarca. The classical work of Starling (1896) invoked the hydrostatic pressure of the fluid in the capillaries, the colloid osmotic pressure of the plasma proteins, and the integrity of the capillary walls as the primary factors involved. This led Epstein (1917) to suggest that the oedema of nephrosis was due to a decrease in the plasma protein level secondary to the albuminuria, with a consequent fall in plasma osmotic pressure. This view was supported by the work of Rennie (1933) but could not be accepted as the complete explanation, for a variety of/

of reasons (Eier, 1954). Current interest in sodium metabolism had interested recent investigators in the part played by this base in promoting nephrotic oedema (Keith, Power and Dougherty, 1950). Sodium retention was envisaged as a compensatory reaction to low plasma osmotic pressure, effected by salt retaining hormones derived from theadrenal glands. That such hormones might in fact produce oedema even in subjects with normal kidney function is exemplified by the effect of administering an excess of desoxycorticosterone acetate to healthy subjects. Levitt and Bader (1951) had shown that both cortisone (17-hydroxy 11-dehydrocorticosterone) and corticotrophin (ACTH) had some sodium retaining activity in the extracellular fluid. Such retention was more pronounced during the first few days of therapy but this period might be followed by sodium and water diuresis if treatment was continued. Furthermore, Addis et al. (1950) had shown that experimental albuminuria in animals was made worse by ACTH or cortisone and improved by adrenalectomy. It had also been firmly established that immediately following an intensive course of either ACTH or cortisone there was a transient depression of endogenous cortisone production by the patient. (Luetscher and Deming, 1950; Kendall, 1951; McIntosh and Holmes, 1951). During this period following cessation of treatment certain "withdrawal phenomena" were known to occur in other diseases such as acute rheumatism, rheumatoid arthritis and eczema, presumably due to a temporary lack of endogenous adrenal 11-oxysteroids. Assuming that similar adrenal

depression/

depression would occur in nephrotic patients to whom hormones were administered it seemed possible that diuresis from loss of sodium, and a lessening of the proteinuria might result during this period.

Cortisone and ACTH were administered to nephrotic patients in large doses in order to test this hypothesis and observations were made on the effects of the hormones and the results of stopping such treatment.

TRIAL OF ACTH AND CORTISONE THERAPY

Fifteen cases of nephrosis admitted to the Royal Hospital for Sick Children, Glasgow, were chosen for this investigation. Each satisfied the following criteria:

(1) Persistent oedema and ascites were present

(2) The urine contained protein exceeding 3 g. per litre (Esbach method)

(3) The serum albumin content was low with consequent reversal of the albumin/globulin ratio and a lowering of the total serum protein content

(4) The blood cholesterol level exceeded 250 mg. per 100 ml. ($CHCl_3$ extraction method)

(5) The body weight had been reasonably stable or increasing during the ten days preceding treatment

(6) The non-protein nitrogen content of the blood was less than 40 mg. per 100 ml.

(7) The systolic blood pressure was less than 120 mm. of mercury and the diastolic pressure less than 90 mm. of mercury (measured by a sphygmomanometer cuff)

Fifteen courses of ACTH and fourteen courses of cortisone were given to these fifteen cases of nephrosis. One case (P.McD.) was treated/

treated with ACTH before oedema developed, because she satisfied the remaining criteria and it was hoped to prevent this sign developing. Throughout the text, first, second and third courses of ACTH are shown thus - (A.1.), (A.2.), (A.3.), ((e.g. E.B. (A.2.)) and similarly the symbols (C.1.), (C.2.), (C.3.) are used to represent successive courses of cortisone. The ACTH cases received a diet containing not more than 88 milli-equivalents of sodium daily and the diet of patients given cortisone contained less than 22 milli-equivalents of sodium per day.

The ACTH was given by intramuscular injection for twelve days, in amounts varying from 40 to 100 mg. daily, together with 2 to 3 g. of potassium chloride by mouth. Cortisone was given in dosage varying from 100 to 300 mg. daily (with a dietary supplement of 2 g. potassium chloride) over a five day period. Eleven courses of cortisone were given intramuscularly and three courses orally.

The patients were weighed each day and the urinary protein concentration was estimated by the Esbach method. In some of the younger patients difficulty in obtaining the urine made collection of the complete 24 hours specimen difficult but this was essayed whenever practicable. Samples of blood were withdrawn before treatment, at the end of treatment and several times during the "withdrawal period" and thereafter.

The total serum protein content was estimated and was fractionated by electrophoresis. The non-protein nitrogen and plasma cholesterol were also estimated as were the erythrocyte sedimentation rate/

rate (E.S.R.) and the packed cell volume (P.C.V.) (Wintrobe method). Throughout the text a diuresis is arbitrarily regarded as polyuria causing fluid loss equivalent to 2 litres of water, or to more than 20 per cent of the body weight of the patient.

ACTH Treatment

The effects of ACTH on the oedema, proteinuria, serum protein level, eosinophil count, blood cholesterol and erythrocyte sedimentation rate will now be discussed under various headings.

Oedema

Variations in oedema are most readily measured by alterations in the body weight of the patients. During the first six days of ACTH therapy the average weights of patients increased as compared to the six days preceding treatment on twelve occasions, but on three occasions the average weight fell. The gain in weight varied from 0.1 kg. to 1.9 kg., the average gain being 0.6 kg. During the second six days of ACTH therapy weight gain occurred in eight cases and weight loss in seven; five of these latter cases subsequently ceased to be oedematous. On no occasion when a case continued to gain weight for 12 days did diuresis subsequently occur. Thus from thirteen courses of ACTH given to eight oedematous patients diuresis resulted in five instances. The weight lost by these five varied from 2.6 kg. to 10.0 kg. (average loss = 6.2 kg.). More detailed information is given in Table XXII in which the average weight of the patient for a series of six day periods, before, during and

TABLE XXII

EFFECTS OF ACTH ON OEDEMA AS SHOWN BY ALTERING BODY WEIGHT

	Average weight for period expressed in Kg.								
	6 Days Before ACTH	1 - 6 Day of ACTH	7 - 12 Day of ACTH	1 - 6 Days After ACTH	6 - 12 Days After ACTH	12 - 18 Days After ACTH	18 - 24 Days After ACTH	Diuresis	Relapse
D.M. (1)	14.0	15.9	15.1	11.4	-	-	11.8	+	o
J.N. (1)	31.5	32.9	32.2	22.0	21.5	21.8	-	+	o
E.B. (1)	35.3	34.8	32.1	28.8	30.6	34.0	38.2	+	+
E.B. (2)	34.0	35.0	36.0 *(32.6)	28.1	27.0	28.0	29.7	+	o
D.McG. (1)	17.4	17.3	16.4	13.6	12.4	12.6	13.0	+	+
D.McG. (2)	16.6	17.0	18.3	19.3	18.9	19.6	19.4	o	
E.M. (1)	17.9	18.5	18.1	18.8	16.8	17.2	16.8	o	
E.M. (2)	16.8	17.8	18.4	16.5	17.0	16.8	16.6	o	
E.M. (3)	19.1	19.8	19.9	20.5	21.5	20.7	-	o	
P.McD. (1)	17.3	17.6	17.8	17.6	17.5	17.6	-	o	
P.McD. (2)	18.5	18.6	18.2	18.4	18.5	18.7	-	o	
C.I. (1)	30.6	30.7	31.1	31.3	31.8	32.9	-	o	
C.I. (2)	33.2	34.8	35.7	35.0	32.7	-	-	o	
H.D. (1)	19.8	21.0	22.5	24.5	26.6	27.1	27.3	o	
A.R. (1)	17.9	16.7	16.5	16.4	16.6	16.6	16.8	o	

* ACTH therapy continued a further 9 days; average weight for this period shown in brackets.

after treatment is shown. In E.B.(A.1.) and D.McG.(A.1.) the gross oedema recurred after 9 and 114 days respectively; in the remaining three cases oedema has remained absent for more than six years ((D.M.(A.1.), J.N.(A.1.), and E.B.(A.2.))

Proteinuria

On fourteen of the fifteen occasions on which ACTH was given the proteinuria increased soon after treatment began and in one case remained unchanged. Diminution of the proteinuria usually occurred 1 to 6 days after hormone treatment had ceased and was unassociated with water loss on seven occasions. In three cases in which diuresis commenced during therapy a marked lessening of proteinuria occurred towards the end of ACTH treatment. In one case (P.McD.) where complete collection of urine was achieved frequently during observation the total daily output of protein doubled when ACTH was given and fell to one half of the original quantity when treatment was stopped. In another case E.B.(A.1.) the daily output of protein (which before treatment with ACTH ranged from 2 to 4 grammes) rose to 4 to 9 grammes in the early days of treatment, and later fell to 0 to 1 gramme per day for the last week of therapy, and the urine remained free of excess protein for 5 years after treatment stopped. This indicates clearly that the fall in proteinuria was not due to dilution of the urine and certainly not due to a fall in plasma albumin which had in fact, altered little during this period. It may therefore be accepted that the administration of ACTH to patients with nephrosis increases proteinuria/

proteinuria for the first few days of treatment and the proteinuria then lessens irrespective of whether ACTH is stopped or not, or whether a diuresis takes place or not. If diuresis takes place before the absolute daily loss of protein lessens, then some dilution of the protein in the urine will inevitably occur. On fourteen occasions proteinuria persisted after treatment and returned to its former gross levels in all these cases except J.N.(A.1.), A.R.(A.1.) and E.B.(A.2.)

Serum Proteins

The total serum protein levels were measured before treatment, after twelve days of ACTH, and sixteen days later. Complete records were obtained on thirteen occasions; the details of such results are shown in Table XXIII. It will be seen that on ten occasions the total protein content per 100 ml. of serum rose by 0.5 g. or more during ACTH therapy and the rise exceeded 1.0 g. in five instances. The total protein level increased slightly in three of the remaining five cases and fell in two.

In twelve cases it was possible to compare the total serum proteins before ACTH treatment began and sixteen days after ACTH treatment stopped. The total serum proteins rose by more than a gramme per 100 ml. on five occasions, by less than one gramme on six occasions, and fell slightly on one occasion. In each of the four cases in whom the most satisfactory diureses had by then occurred ((D.McG.(A.1.), J.N.(A.1.), E.B.(A.2.) and D.M.(A.1.)) the level of total serum proteins after treatment exceeded the level before treatment by at least/

TABLE XXIII

EFFECTS OF ACTH ON N.P.N. AND SERUM PROTEINS

	Non-Protein Nitrogen (mg. per 100 ml.)			Total Serum Proteins (g. per 100 ml.)			Albumins (g. per 100 ml.)			Globulins (α, β & γ) (g. per 100 ml.)				
	Before ACTH	At End of ACTH	16 Days After ACTH	Before ACTH	At End of ACTH	16 Days After ACTH	Before ACTH	At End of ACTH	16 Days After ACTH	Before ACTH	At End of ACTH	16 Days After ACTH	Diuresis	Relapse
D.M. (1)	26	28	18	4.09	4.71	6.59	0.91	1.21	5.06	3.18	3.50	1.53	+	o
J.N. (1)	21	25	33	3.06	3.65	4.30	0.28	1.13	1.36	2.78	2.53	2.94	+	o
E.B. (1)	34	26	27	3.69	4.14	3.51	0.48	0.45	0.28	3.21	3.69	3.23	+	+
E.B. (2)	27	26	21	3.84	5.78	7.49	0.13	2.72	2.90	3.70	3.06	4.59	+	o
D.McG. (1)	28	35	33	4.50	4.34	5.96	0.75	0.44	3.96	3.75	3.90	2.00	+	+
D.McG. (2)	32	19	-	4.29	4.07	-	0.93	0.75	-	3.34	3.32	-	o	
E.M. (1)	31	45	21	3.92	4.99	4.06	0.67	0.48	0.57	3.25	4.51	3.49	o	
E.M. (2)	21	25	-	4.06	5.70	-	0.57	1.66	-	3.49	4.03	-	o	
E.M. (3)	-	25	-	-	4.65	-	-	0.61	-	-	4.04	-	o	
P.McD. (1)	20	18	21	5.41	6.14	5.45	2.92	2.52	2.64	2.49	3.62	2.81	o	
P.McD. (2)	21	22	21	5.45	7.67	5.88	2.64	4.06	3.20	2.81	3.61	2.68	o	
C.I. (1)	37	39	31	3.85	4.10	4.01	0.92	0.50	0.64	2.93	3.60	3.44	o	
C.I. (2)	31	28	28	4.01	5.21	5.71	0.64	0.55	0.70	3.39	4.67	5.01	o	
H.D. (1)	29	23	26	3.76	4.12	4.26	0.58	0.30	1.20	3.18	3.82	3.06	o	
A.R. (1)	30	24	23	5.02	5.71	5.18	1.39	1.27	2.71	3.63	4.44	2.37	o	

least 1 g. In E.B.(A.1.) and A.R.(A.1.), who each had a transient diuresis, the total serum protein level rose during ACTH therapy and fell after it had been stopped. It is interesting that in two oedematous patients in whom a marked rise in proteins was noted during and after ACTH treatment, diuresis did not occur.

The various components of the serum protein content were measured and these results are shown in Table XXIII. The α, β and γ globulins were estimated separately, but at this time (1951) no significant differences were noted in the behaviour of these globulins which are shown as a total.

Several interesting points arose when the alterations in serum albumin levels were studied. In fourteen instances the levels immediately before and after ACTH treatment were estimated; on five occasions a rise in the level of serum albumin arose during treatment and by this time diuresis had occurred in two of these cases; in the nine remaining cases a decrease occurred in the level of plasma albumin. Sixteen days after ACTH treatment an increase exceeding 1 gramme per 100 ml. had occurred in the serum albumin level of five cases and in four of these diuresis had already begun.

The alterations in serum globulin were also of interest. In three of the thirteen instances when complete results were obtained the total globulins were markedly lower sixteen days after ACTH treatment than before. In two of these cases diuresis had occurred and in all three a marked increase in serum albumin was noted. In C.I.(A.2.), one case in which the total serum protein

level rose and no diuresis had occurred, a marked increase in serum globulin level explained this rise.

All these results indicate that ACTH can cause a disappearance of the oedema without material alteration in the albumin or globulin fraction or the total serum protein levels; that a rise in the serum albumin is not always associated with diuresis ((E.M.(A.2.)); and that a marked rise in serum globulins did not induce diuresis ((C.I.(A.2.)). Nevertheless the cases in whom the best results were obtained were those in whom the greatest increases in serum albumin occurred in the sixteen days after treatment ((D.M.(A.1.), D.McG.(A.1.), J.N.(A.1.), E.B.(A.2.)).

Other Observations

Serial eosinophil counts were completed on eleven occasions and very marked falls occurred during ACTH therapy; in all cases except P.McD.(A.2.) the levels returned to normal when treatment was stopped. During ACTH therapy the plasma cholesterol level rose in four cases and fell in nine (Table XXIV); after treatment the level was lower in ten of the cases where comparison was possible and higher in two. In all five cases in whom diuresis occurred the cholesterol level was lower sixteen days after ACTH treatment than before and in four cases had fallen to below 250 mg. per 100 ml. The packed cell volume was estimated before, during and after treatment with ACTH but little alteration occurred in any case.

This implies that the fluctuations in total serum proteins, albumin/

TABLE XXIV

EFFECTS OF ACTH ON PLASMA CHOLESTEROL, CIRCULATING EOSINOPHIL COUNT, ERYTHROCYTE SEDIMENTATION RATE AND PACKED CELL VOLUME (WINTROBE)

	Plasma Cholesterol (mg. per 100 ml.)			Eosinophils (per cu.mm.)			Erythrocyte Sedimentation Rate (mm./hr.)			Packed Cell Volume (%)				
	Before ACTH	At End of ACTH	16 Days After ACTH	Before ACTH	At End of ACTH	16 Days After ACTH	Before ACTH	At End of ACTH	16 Days After ACTH	Before ACTH	At End of ACTH	16 Days After ACTH	Diuresis	Relapse
D.M. (1)	388	596	72	-	-	-	55	32	34	39	44	41	+	o
J.N. (1)	241	208	208	-	-	-	52	-	32	40	-	40	+	o
E.B. (1)	557	554	438	236	24	209	40	47	50	41	44	42	+	+
E.B. (2)	470	436	160	188	-	326	55	39	32	32	34	30	+	o
D.McG. (1)	486	220	224	216	64	242	45	35	49	41	41	42	+	+
D.McG. (2)	410	660	-	220	44	-	36	22	-	46	45	-	o	
E.M. (1)	296	460	411	268	96	232	49	45	40	42	40	41	o	
E.M. (2)	411	351	-	232	88	-	40	54	-	41	39	-	o	
E.M. (3)	-	368	-	-	132	-	-	25	-	-	45	-	o	
P.McD. (1)	334	228	229	226	16	178	44	-	-	41	-	-	o	
P.McD. (2)	229	172	182	178	330	242	45	35	42	38	39	42	o	
C.I. (1)	473	733	731	286	-	362	45	28	28	47	47	48	o	
C.I. (2)	731	654	459	326	11	286	38	41	19	41	41	42	o	
H.D. (1)	630	400	460	242	44	242	25	13	40	41	43	41	o	
A.R. (1)	513	259	335	-	-	-	31	18	45	41	41	42	o	

albumin, globulins and cholesterol cannot be explained away by alteration of the total plasma volume unless the total red blood cell volume altered pari passu . The erythrocyte sedimentation rate was high in all cases and was not altered by treatment except in the two cases rendered asymptomatic.

Effects of Cortisone Treatment

Oedema

An increase of more than 0.5 kg. in the weight of the patient during cortisone therapy as compared to the preceding six days occurred on nine occasions, the maximum weight gained being 2.7 kg. (Table XXV). Three cases did not gain weight and in each of these cases marked diuresis occurred subsequently. Diuresis occurred in six of the eleven cases who gained weight during cortisone therapy. In each case the marked loss of fluid occurred two to twelve days after the cortisone had been stopped.

This weight loss ranged from 2.7 kg. to 9.4 kg. and represented a loss of up to 57 per cent. of the final body weight of the patient. Diuresis occurred in nine children and after a period varying from 12 to 78 days, but the oedema returned on seven occasions. In R.P.(C.1.) and A.P.(C.1.) oedema had not recurred eight years later. Diuresis did not occur in five cases and in each instance the oedema was increased by the treatment.

Proteinuria

On every occasion the proteinuria increased during cortisone therapy/

TABLE XXV

EFFECTS OF CORTISONE ON OEDEMA AS SHOWN BY ALTERING BODY WEIGHT

	Average Weights (Kg.) for Periods Stated								
	Six Days Before Cortisone	Five Days on Cortisone	1 - 6 Days After Cortisone	7 - 12 Days After Cortisone	13-18 Days After Cortisone	19-24 Days After Cortisone	25-30 Days After Cortisone	Diuresis	Relapse
R.P. (1)	13.8	13.5	12.2	11.1	11.1	11.2	11.5	*	o
A.P. (1)	13.2	13.2	13.2	11.1	10.5	10.6	10.8	*	o
J.N. (1)	28.5	28.3	28.0	26.4	20.2	19.5	19.6	*	*
J.H. (1)	18.2	19.3	19.5	15.9	13.0	15.1	15.8	*	*
J.H. (2)	14.6	15.4	16.4	13.3	12.5	13.0	-	*	*
E.B. (1)	35.1	37.8	39.9	32.3	30.5	-	-	*	*
E.B. (2)	31.0	33.1	35.5	33.7	29.3	31.0	-	*	*
E.B. (3)	34.3	35.2	34.2	28.4	31.0	31.5	-	*	*
H.D. (1)	27.0	27.2	27.4	25.8	19.7	18.3	18.8	*	*
C.Y. (1)	23.2	23.5	25.0	25.8	27.7	25.6	-	o	-
F.M. (1)	27.5	28.6	29.1	28.6	29.7	-	-	o	-
F.M. (2)	20.8	21.4	23.1	23.6	24.4	24.2	26.5	o	-
D.N. (1)	13.6	14.6	15.5	16.2	16.8	17.0	-	o	-
D.N. (2)	17.4	17.9	19.5	18.5	18.6	18.3	-	o	-

therapy and fell below the original level 36 to 72 hours after the hormone was stopped. That this reduction was due to decreased daily loss of protein and not to dilution of the urine due to water diuresis was apparent from the marked fall from 16 g. protein per litre to less than 0.5 g. protein per litre which occurred in one case with no parallel increase in urinary output. This finding was more accurately confirmed in E.B.(C.1., C.2. and C.3.) where total daily urinary output was measured and the total quantity of protein lost daily assessed. Proteinuria returned on twelve occasions after a period varying from 3 to 56 days. In the two remaining cases proteinuria has not returned after eight years ((A.P.(C.1.) and R.P.(C.1.)).

Serum Proteins

The effect of five days' treatment with cortisone on the total serum protein level was observed on ten occasions. On two occasions the total serum protein level rose and on five occasions fell. Two to four weeks after the cortisone was stopped the total serum proteins had risen in ten of these cases, the rise varying from 0.1 g. to 1.8 g. A small diminution had occurred in the remaining two cases. These measurements were made in seven of the nine cases in whom diuresis later occurred; the level of total serum protein rose in all seven cases, by varying amounts (range = 0.1 g. to 1.8 g. per 100 ml.) On five occasions ((A.P.(C.1.), R.P.(C.1.), E.B.(C.2.), E.B.(C.3.), H.D.(C.1.) and F.M.(C.2.)) the proteins had risen by 1 g. per 100 ml. or more and in the first three a diuresis had by then occurred. Three months/

months after treatment two children ((R.P.(C.1.)) and ((A.P.(C.1.)) showed further increase in their total serum protein content but in all others except F.M. (C.1.) the serum proteins had fallen to their original low levels. The various components of the serum proteins were measured and these are shown in Table XXVI. The α, β and γ fractions of the globulin were separated but, as no significant individual alteration was noted at this time they were shown as "total globulins". It will be seen that during cortisone therapy the level of serum albumin fell on seven occasions and rose on three. Two to four weeks later the albumin had risen by more than 0.5 g. in five cases and in all five children diuresis had occurred by this time. Thus the overall pattern in cases responding to cortisone treatment was a fall in serum albumin during treatment followed later by a much larger rise.

When the alterations in the levels of serum globulins are reviewed it may be seen that, while treatment was being given a rise occurred in four cases and a fall in six cases. Diuresis later occurred in four of the six cases whose serum globulin had fallen and in two of the four cases which showed a rise in total serum globulins. Two to four weeks after treatment six out of the twelve cases had a lower level of serum globulins and by then diuresis had occurred in four of these. This fractionation shows clearly that the alteration which occurs in serum protein pattern, when cortisone produces a diuresis is, essentially, a return towards normal in the albumin content of the serum with a synchronous fall in total globulin content. It/

TABLE XXVI

[redacted]

EFFECTS OF CORTISONE ON N.P.N. AND SERUM PROTEINS

	Non-Protein Nitrogen (mg. per 100 ml.)			Total Serum Proteins (g. per 100 ml.)			Serum Albumin (g. per 100 ml.)			Serum Globulins (g. per 100 ml.)				
	Before Cortisone	At End of Cortisone	16 Days After Cortisone	Before Cortisone	At End of Cortisone	16 Days After Cortisone	Before Cortisone	At End of Cortisone	16 Days After Cortisone	Before Cortisone	At End of Cortisone	16 Days After Cortisone	Diuresis	Relapse
R.P.(1)	34	-	22	3.72	-	5.02	0.58	-	2.27	3.12	-	2.75	+	o
A.P.(1)	32	21	34	4.14	3.80	5.34	0.30	0.22	2.85	3.82	3.57	2.49	+	o
J.N.(1)	31	45	26	5.17	-	5.24	0.31	-	0.48	4.84	-	4.76	+	+
J.H.(1)	31	39	-	4.29	3.41	-	0.27	0.21	-	4.01	3.17	-	+	+
J.H.(2)	-	36	31	-	5.12	5.62	-	0.37	2.46	-	4.74	3.13	+	+
E.B.(1)	27	26	20	3.51	3.78	4.02	0.28	0.80	0.80	3.24	2.99	3.18	+	+
E.B.(2)	21	32	21	4.02	3.74	5.88	0.80	0.46	1.53	3.28	3.27	4.33	+	+
E.B.(3)	32	21	20	4.89	4.8	5.88	1.18	0.48	1.25	3.71	4.49	4.62	+	+
H.D.(1)	23	27	19	3.02	3.49	5.79	0.17	0.33	1.68	2.84	3.16	4.14	+	+
C.Y.(1)	21	29	23	4.80	4.82	4.29	1.15	0.40	0.80	3.65	4.34	3.50	o	-
F.M.(1)	21	29	23	4.65	-	5.42	0.95	-	0.50	3.66	-	4.92	o	-
F.M.(2)	23	36	24	5.71	3.44	4.84	0.93	0.45	1.17	4.75	2.98	3.68	o	-
D.N.(1)	25	22	28	4.18	3.7	4.38	0.34	1.62	0.39	3.84	2.08	3.99	o	-
D.N.(2)	28	29	26	4.31	4.92	5.17	0.32	0.30	0.31	3.99	4.60	4.86	o	-

It should be emphasised that the typical electrophoretic protein pattern, as shown in all these cases before treatment, is not specific for nephrosis but occurs after burns, acute infections or trauma (Teilum, Angback, Harboe and Simonsen, 1951).

Having ascertained the quantity of each of these components of the serum proteins per unit volume of blood an attempt was then made to determine the total quantity of each protein in circulation. To this end the blood volume was estimated before and after treatment in four cases, two of whom responded by diuresis. From these values it was possible to calculate the absolute circulating quantities of each fraction (Table XXVII). It will be seen that the marked drop in blood volume which occurred in R.P.(C.1.) and J.N.(C.1.) who responded to cortisone, tended to mask the absolute fall in globulin (measured per unit volume) whilst the actual level of the albumin was, in the same way, exaggerated. Nevertheless, the final level of circulating albumin in R.P.(C.1.) amounted to 13.4 g., (the original level had been only 3.8 g.). This case responded by diuresis and neither oedema nor albuminuria has recurred. In the two cases who failed to respond by diuresis ((C.Y.(C.1.) and F.M.(C.1. and C.2.)) a fall in albumin and rise in fibrinogen occurred during treatment.

Other Observations

During cortisone therapy a marked rise in the cholesterol content of the blood occurred in one patient on three occasions, a marked fall in three cases and little change occurred in the remaining five cases observed. After treatment was stopped a fall occurred

in/

TABLE XXVII

EFFECTS OF CORTISONE TREATMENT ON PLASMA VOLUME AND TOTAL CIRCULATING PROTEINS

	R.P.		J.N.		C.Y.			F.M.		
	Before Cortisone Treatment	After Diuresis	Before Cortisone Treatment	After Diuresis	Before Cortisone Treatment	At End of Cortisone Treatment	2 Weeks After Cortisone	Before Cortisone Treatment	At End of Cortisone Treatment	2 Weeks After Cortisone
Plasma Volume (ml.)	643	591	1360	1042	908	979	930	1069	1086	875
Total Proteins Circulating (g.)	24.6	30.3	72.9	57.4	44.3	50.8	36.8	62.9	40.1	38.8
Total Albumin Circulating (g.)	3.8	13.4	4.3	5.0	10.4	4.7	6.1	9.6	5.5	6.9
Total Globulins in Circulation (g.)	17.5	13.3	60.2	40.2	27.3	35.4	24.2	42.8	25.9	25.2
Total Globulin Circulating (g.)	2.6	2.9	5.8	9.4	5.7	7.1	4.4	8.6	4.3	2.7
Total Fibrinogen Circulating (g.)	0.7	0.7	2.6	2.8	0.9	3.6	2.1	1.9	4.4	4.0

in the cholesterol level, as compared to the original value, on thirteen occasions and a rise occurred in one case only. In those cases who relapsed after treatment and in the cases who did not respond by diuresis, the cholesterol returned to the high initial levels. In A.P. and R.P. on the other hand, the levels fell to normal and 8 years after treatment have remained there. Fuller details are given in Table XXVIII. Serial eosinophil counts were performed on thirteen occasions: during treatment the level fell in twelve cases and rose in one ((J.H.(C.2.)). After treatment a rapid rise to normal levels was observed in all cases. The erythrocyte sedimentation rate was elevated to very high levels in all five cases in which the measurement was made and was altered but little by treatment. Packed cell volume measurements were made on ten cases and again showed no alteration in plasma volume which would account for the altering biochemical findings.

Comparison of the Effects of ACTH and Cortisone Treatment

In this series diuresis occurred in oedematous nephrotics on nine occasions after fourteen courses of cortisone (64% success); and on five occasions by thirteen courses of ACTH (38% success). The extent of the diuresis is best judged by expressing the weight of oedema lost as a percentage of the final body weight, and this is done in Table XXIX. It will be seen that the weight loss in each treatment group varied from 20 per cent. to about 55 per cent. of the final body weight and the duration of the loss of oedema did not differ/

TABLE XXVIII

EFFECTS OF CORTISONE ON PLASMA CHOLESTEROL, EOSINOPHIL LEVEL, PACKED CELL VOLUME AND ERYTHROCYTE SEDIMENTATION RATES (WINTROBE)

	Plasma Cholesterol (mg. per 100 ml.)			Eosinophils (per cu.mm.)			Erythrocyte Sedimentation Rate (mm./hr.)			Packed Cell Volume (%)				
	Before Cortisone	At End of Cortisone	16 Days After Cortisone	Before Cortisone	At End of Cortisone	16 Days After Cortisone	Before Cortisone	At End of Cortisone	16 Days After Cortisone	Before Cortisone	At End of Cortisone	16 Days After Cortisone	Diuresis	Relapse
R.P. (1)	430	-	350	264	156	196	-	-	-	-	-	-	+	o
A.P. (1)	620	239	444	262	168	206	-	-	-	-	-	-	+	o
J.N. (1)	580	-	528	242	88	308	-	-	-	-	-	-	+	+
J.H. (1)	527	446	462	308	176	264	-	-	-	-	-	-	+	+
J.H. (2)	554	484	472	154	210	320	-	-	-	-	-	-	+	+
E.B. (1)	438	733	377	209	106	312	50	40	43	42	41	42	+	+
E.B. (2)	460	723	388	312	182	126	51	50	-	41	40	42	+	+
E.B. (3)	388	710	662	-	-	-	-	-	-	-	-	-	+	+
H.D. (1)	315	447	169	242	126	308	47	44	42	45	43	44	+	+
C.Y. (1)	368	320	272	218	88	294	-	-	-	-	-	-	o	-
F.M. (1)	496	-	430	242	110	363	-	-	-	-	-	-	o	-
F.M. (2)	303	302	204	326	186	248	-	-	-	-	-	-	o	-
D.N. (1)	670	672	436	260	154	220	56	25	45	34	37	38	o	-
D.N. (2)	315	447	169	286	22	132	45	35	65	38	37	36	o	-

TABLE XXIX

A Comparison of the Diuretic Effect of ACTH and Cortisone

A C T H			CORTISONE		
Patient	Weight After Diuresis (kg.)	% Loss in Weight by Diuresis	Patient	Weight After Diuresis (kg.)	% Loss in Weight by Diuresis
D.M.(1)	11.0	27%	R.P.(1)	10.5	31%
J.N. (1)	20.2	55%	A.P.(1)	10.4	27%
E.B. (1)	28.5	25%	J.N.(1)	19.1	46%
E.B. (2)	27.2	23%	J.H.(1)	13.4	36%
D.McG.(1)	11.9	48%	J.H.(2)	12.2	20%
			E.B.(1)	29.2	20%
			E.B.(2)	29.0	26%*
			E.B.(3)	28.0	23%
			H.D.(1)	17.5	57%
Mean	19.8	36%	Mean	18.8	32%

* The second course of Cortisone given to E.B. when his weight was rising sharply as oedema increased after his first diuresis following Cortisone.

differ significantly in the two groups. Two of the cortisone treated cases and two of the ACTH treated cases have had apparently permanent remissions but such occurrences might have been fortuitous. The two hormones appear to produce a diuresis similar in extent and duration but cortisone seemed to be the hormone of choice, and cortisone promotes diuresis in a larger proportion of cases.

A striking point of difference in the two groups was the time of onset of diuresis. This did not occur in any of the cortisone treated/

treated group until after the administration of the hormone had ceased whereas in all the cases treated successfully with ACTH diuresis started during the administration of the hormone although an acceleration of the diuresis occurred when ACTH treatment was stopped. Since a five day course of cortisone was given on each occasion in this series it was not possible to say that diuresis would not have occurred during cortisone therapy had this been prolonged to 12 days. This point had been investigated by Luetscher (1951) and it appeared at that time that diuresis did not occur during the administration of cortisone. It should be emphasised that although diuresis was provoked on fourteen occasions, only in five instances did loss of oedema persist more than three months.

In both groups the proteinuria became aggravated during treatment and decreased rapidly as a rule when hormone therapy was stopped. On three occasions during ACTH treatment, however, the proteinuria lessened sharply and this finding may be explained by the work of Lauson et al. (1954). In every instance but four (that is, on twenty-five occasions) the proteinuria later returned to the former gross levels. It appears that treatment with either ACTH or cortisone might temporarily affect the proteinuria in some way which was apparently unconnected with diuresis. This fact was encouraging since it suggests that these hormones might have some influence, however indirect, on the basic cause of this disease: their action being more complex than a simple diuretic.

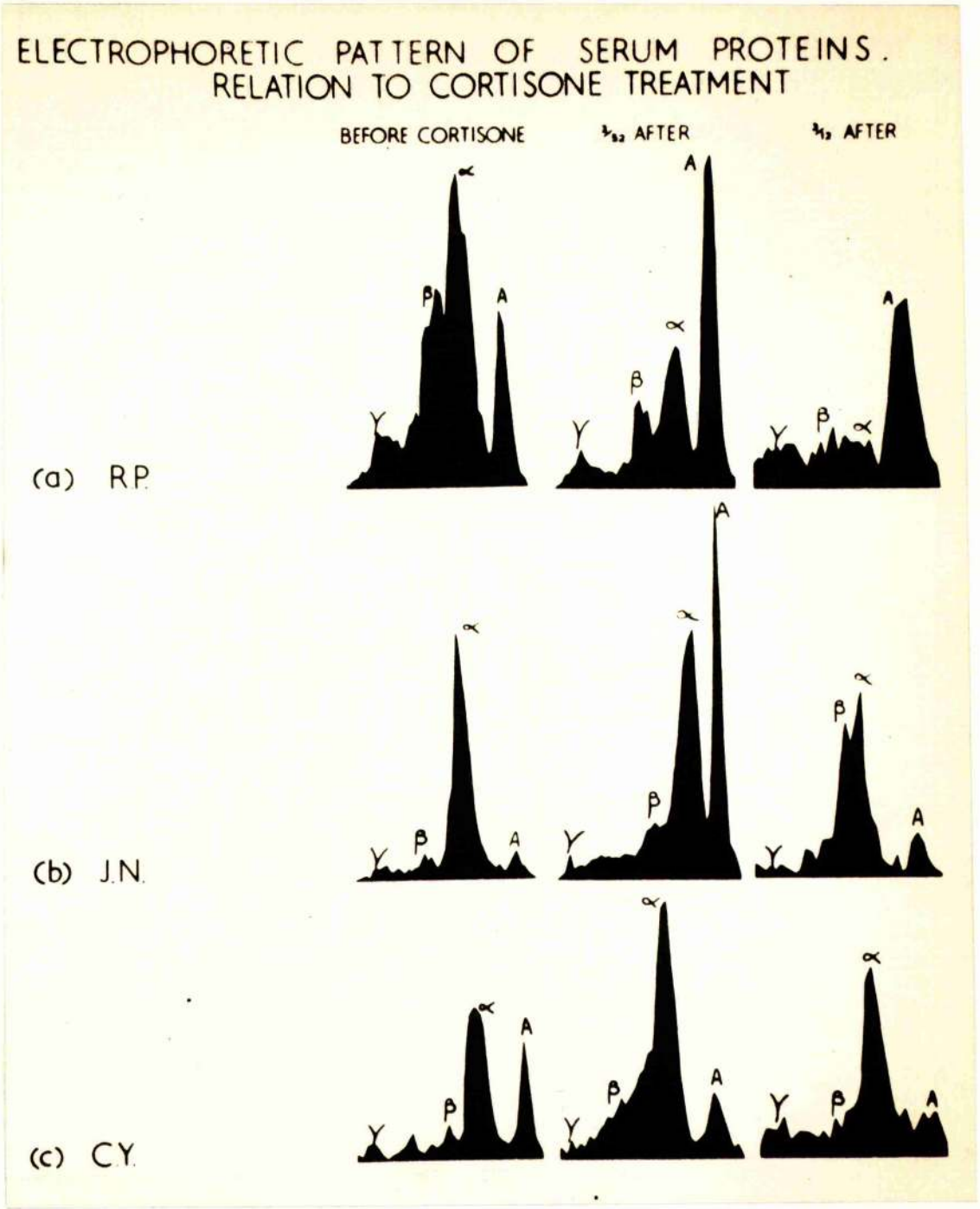
Similar/

Similar alterations in serum proteins were produced by both hormones, the tendency being to return the pattern towards normal after treatment; that is, to increase the serum albumin level and reduce that of the serum globulins. The rise in level of albumin tended to be most marked in cases who had responded by diuresis. Figures LXIII and LXIV show clearly the similarity of the response of the serum proteins to cortisone and ACTH respectively. In Figure LXIII are shown the patterns of three cases; R.P.(C.1.) who had a diuresis and is apparently cured, J.N.(C.1.) who had a transient diuresis and C.Y.(C.1.) who had no response. The first had a marked and persistent elevation of the serum albumin level after treatment, and the second a transient elevation of serum albumin content; the third no such response. Figure LXIV demonstrates a similar sequence of events in cases responding completely, temporarily and not at all to ACTH ((D.M.(A.1.), D.McG.(A.1.), E.M.(A.1.)).

It appears quite certain that the diuresis precipitated by ACTH or cortisone is not dependent on alterations in the serum proteins and may occur quite separately. This is clearly shown by the case of J.N.(C.1.) after cortisone treatment. In this boy the oedema and proteinuria had both disappeared although the total circulating protein had fallen from 72.9 g. to 57.4 g. and the <u>total circulating</u> albumin had risen by only 0.7 g. Furthermore, the fact that the albumin level in this boy's serum did not rise, despite the cessation of proteinuria, is strongly suggestive of

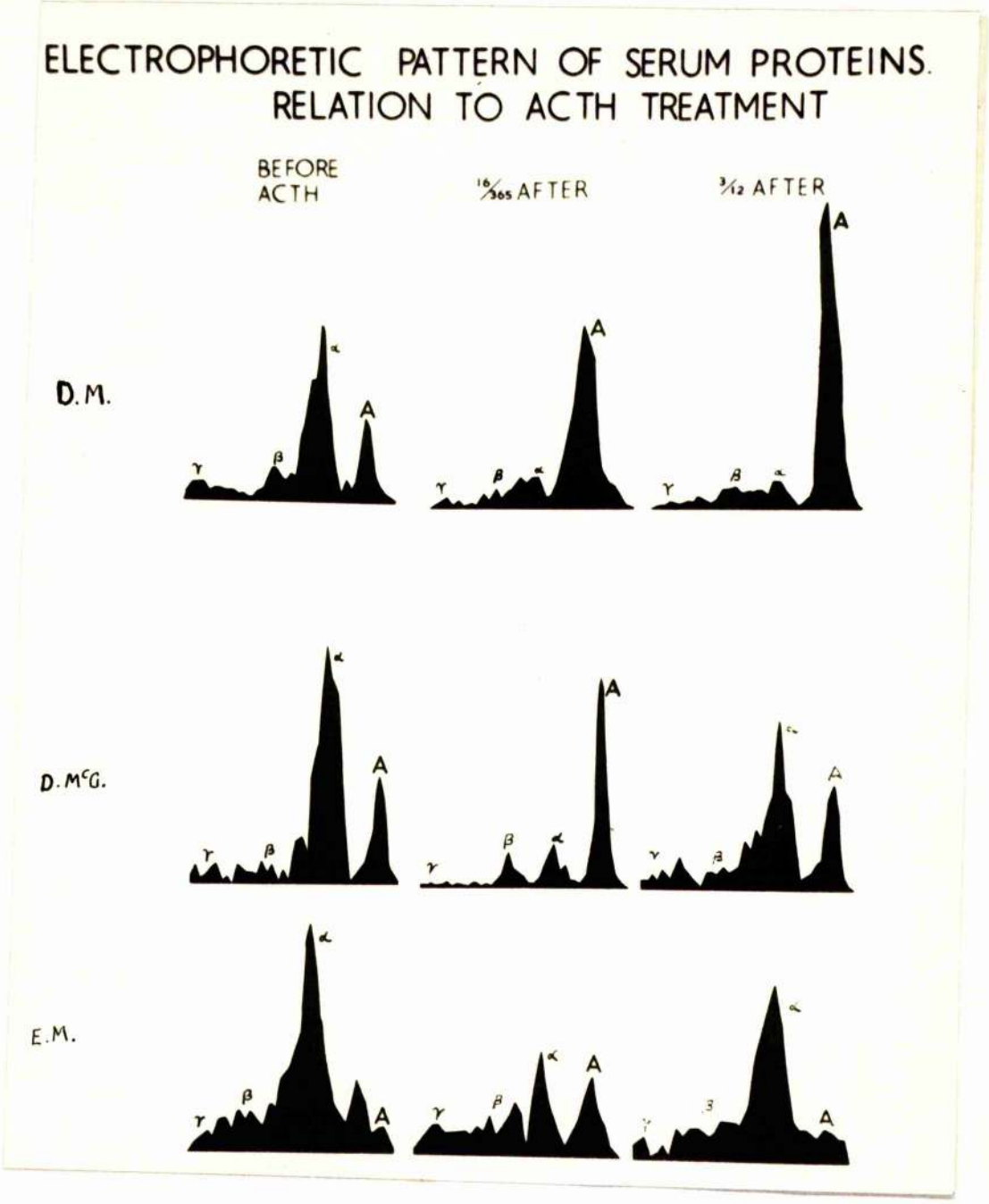
some/

FIGURE LXIII



A = Albumin
α = Alpha Globulin
β = Beta Globulin
γ = Gamma Globulin

FIGURE LXIV



A = Albumin
α = Alpha Globulin
β = Beta Globulin
γ = Gamma Globulin

some factor modifying albumin metabolism unless it were being lost elsewhere. The effects of ACTH and cortisone on the cholesterol level of the plasma were essentially similar. During hormone therapy a rise occurred in the blood cholesterol level of some cases in each group and in the others a fall, but in both groups a considerable fall occurred where diuresis arose, followed by a return to the former high levels if the nephrotic state worsened again (Arneil and Wilson, 1952, 1953). These findings only partly agree with the results of Conn et al. (1950) who suggested that the level of plasma cholesterol was depressed by ACTH therapy (due to a fall in cholesterol esters, used to form cortisone). Adlersberg et al. (1950) suggest that cortisone therapy has a "sparing effect" on cholesterol esters and causes a rise in plasma cholesterol. They also noted hypercholesterol-aemia when ACTH was given over a period which appears contradictory to the findings of Conn quoted above. Wolfson et al. (1950) have obtained evidence to suggest that such a rise in cholesterol on ACTH therapy may result from a depression of the activity of the thyroid gland. In both groups an eosinopenia developed during therapy and disappeared when the hormones were stopped. This eosinopenic response to therapy was more marked in the ACTH group. In neither group was the packed cell volume or the erythrocyte sedimentation rate influenced by treatment.

Toxic/

Toxic Effects of Cortisone and ACTH Treatment

The toxic effects resulting from these hormones are worthy of note. In the cortisone group low grade cellulitis (common in any nephrotic patient) occurred in two cases and these infections were easily controlled by penicillin. In the ACTH group sterile abscesses developed in three cases, large, fluctuating, painless, sloughs forming insidiously and painful induration at the sites of injection were common. Precordial pain was complained of by two patients, diarrhoea occurred on one occasion and faecal impaction in one case. A major complication occurred in E.B.(A.2.) who suddenly developed status epilepticus on his nineteenth day of ACTH therapy; (80 mg. daily). A lumbar puncture was performed and the cerebro-spinal fluid was found to be normal. Marked hypokalaemia was found to exist (serum potassium = 3.2 m.eq. per litre) despite the fact that 3 g. potassium chloride had been given daily throughout the administration of the hormone. Electrocardiographic tracings confirmed this finding and both serum level and electrocardiograph were rapidly restored to normal when potassium chloride was administered by gastric tube. Despite adequate and prolonged sedation with intramuscular paraldehyde the boy continued in deep coma, with intermittent convulsions for 72 hours; throughout this period no hypertension was present. He gradually returned to consciousness but has now a psychopathic personality/

personality reminiscent of the post-encephalitic state. E.B. had been an intelligent boy, eight years of age, and had no history of having a fit prior to this incident. The advent of convulsions in patient receiving ACTH or cortisone has been recorded on several occasions in the United States of America. Sprague (1951) mentions four such cases and quotes two others who progressed to coma and died. The post mortem finding (in one of these cases) consisted of degeneration of the cells of the cerebral cortex; an ominous portent. Another fatal case was recorded by Geppert et al. (1952); this occurred during cortisone treatment and at post mortem revealed only congestion of the brain with no abnormalities of the neurons and a terminal bronchopneumonia. Other cases have been recorded by Lowell et al. (1951) and Dorfman et al. (1951). Such cases were noted to have hypokalaemia and alkalosis as had our case but one must conclude that some direct action of the hormones on the biophysiology of the cells of the cerebral cortex is involved. It appears that E.B. was fortunate to recover, after lapsing into coma of a type which has previously proved fatal.

THE MECHANISM OF STEROID-INDUCED DIURESIS

When considering possible mechanisms by which ACTH and cortisone might relieve the anasarca of nephrosis in 1954 it was first essential to visualise the process of oedema formation in/

in this disease. Whatever the pathogenesis of nephrosis might be, the occurrence of gross proteinuria due to an organic or functional lesion of the nephron was indisputable. That such proteinuria was partly responsible for the fall in plasma albumin level had never been seriously questioned, although diminution of hepatic synthesis of albumin had been suggested as a complementary factor. A fall in the level of albumin in the plasma causes a marked decrease in the osmotic pressure of the latter. In these 15 cases the average osmotic pressure exerted by the serum proteins was only 8.6 mm. Hg. as opposed to the average figure of 30.9 mm. Hg. obtained by Govaert's formula as employed by Rennie (1933). The lowered osmotic pressure results in compensatory retention of sodium and chloride (Gamble, 1949) and oedema becomes apparent. This is probably due to a failure of the plasma to uplift the extravascular extracellular fluid in consequence of diminished plasma osmotic pressure (as Starling's hypothesis suggests). As a result, whatever the pathogenesis may be, water, sodium and chloride must be retained (Emerson et al. 1951) and this is effected by an increased activity of adreno-corticoid hormones (Deming and Luetscher, 1950) and of vasopressin from the posterior pituitary gland (Robinson and Farr, 1940; Lauson et al. 1952). These hormones act on the renal tubules and first produce and then maintain the saline-logged state of the nephrotic. Any process which interferes with the production or balance of these hormones may lead

to/

to loss of sodium, water and oedema. If the basic process of proteinuria and hypo-albuminaemic persists, loss of oedema of this type will last for as long as the diminution in hormones retaining salt and water persists, and oedema will then return.

The impact of cortisone and ACTH on this abnormal electrolyte balance in oedematous patients, the resultant of the interaction of these hormones was then considered.

It had been shown beyond any reasonable doubt, that the administration of cortisone in large doses causes depression and atrophy of the adrenal cortex (Sprague, 1951; McIntosh and Holmes, 1951) which results in a deficiency in hormone output on stopping cortisone. A cessation of the excretion of ACTH by the anterior lobe of the pituitary affects the zonae reticularis et fasciculata but not the zona glomerulisa of the adrenal cortex (Deane and Greep, 1946). It seemed important that this atrophy of the adrenal cortex probably resulted in diminished output of not only the 11-oxysteroids, but all other cortical hormones as well. Thus a diminished output of 17-ketosteroids can be shown in the urine and a beneficial effect occurs in cases of the adrenogenital syndrome. (Bishop et al. 1952). A diminished output of cortisone in response to ACTH may be demonstrated during this period by the poor eosinopenic response to ACTH administration. The loss of sodium and chloride presumed to result from diminished activity of the salt retaining hormones has been clearly demonstrated by Luetscher (1951).

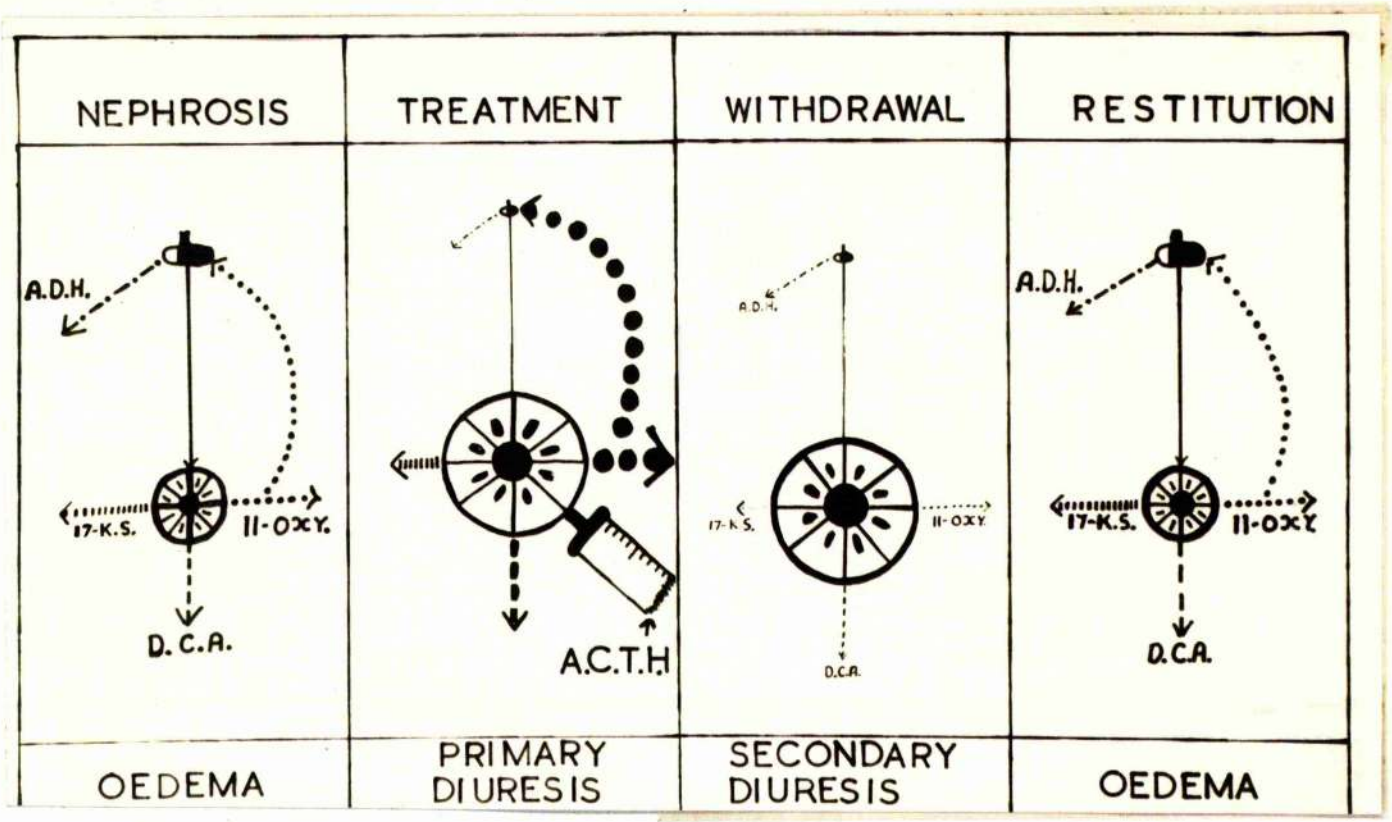
Desoxycorticosterone/

Desoxycorticosterone acetate is fifty times as potent in retaining sodium as cortisone and it seemed likely that a human equivalent of this synthetic substance must exist and be affected by adrenal depression. (A new corticoid substance has been described by Reichstein (1953) which is present in beef adrenal. The work of Tait, Simpson and Grundy (1952) has postulated the presence of a similar substance which they named electrocortin but it was Reichstein who finally identified it as 16-hydroxy 11-desoxycorticosterone). It seems virtually certain that during the days succeeding the sudden stopping of cortisone or ACTH treatment the adrenal output of all these hormones is markedly diminished until adrenal function returns. It is in this critical period that the loss of sodium, chloride and water occurs and may cause reduction in the oedema of nephrotic patients. This conception of diuresis following ACTH or cortisone treatment is shown in Figures LXV and LXVI.

It seemed possible that this mechanism might play a part in many of the well-known methods of producing diuresis. Such forms of stress as acute pneumococcal infection, measles, malaria, burns, scalds or the administration of nitrogen mustards all provoke an outflow of adrenocortical hormones, followed by a sudden decrease and possibly a consequent diuresis. It had been known for many years that diuresis does in fact occur after such acute incidents and the above explanation appeared satisfactory. The intravenous infusion of concentrated plasma, human albumin and plasma substitutes, can only result in a very/

FIGURE LXV

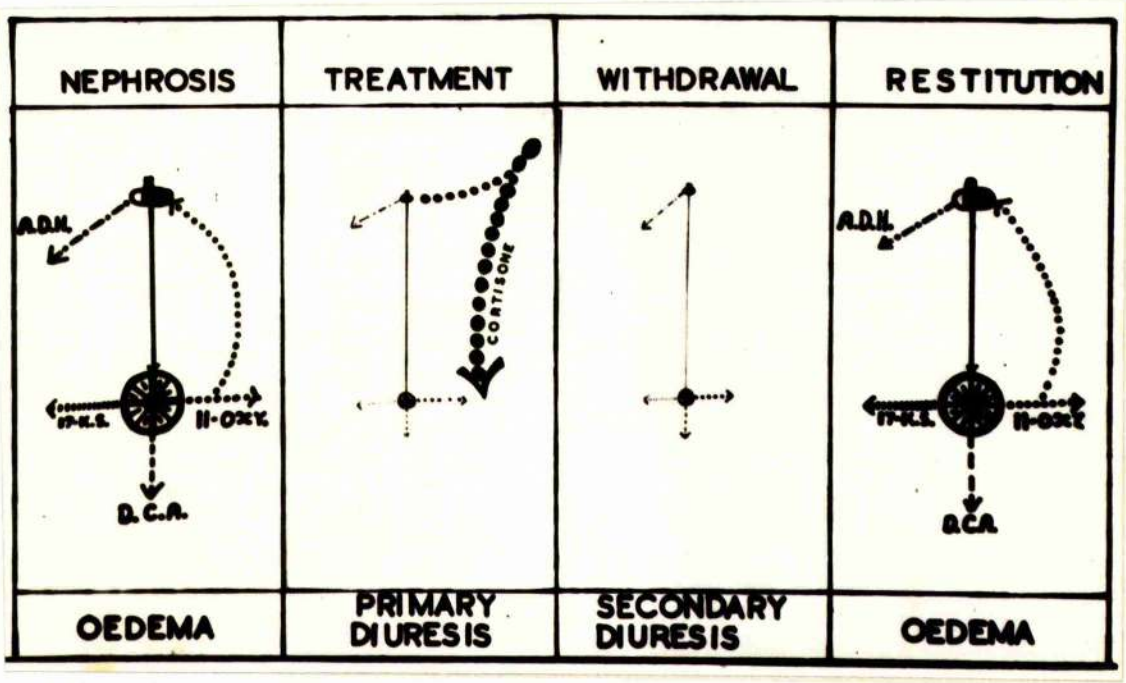
Postulated Causes of Diuresis in Patients Treated by A.C.T.H.



Vertical Axis = Pituitary Gland - Adrenal Cortex
D.C.A. :- aldosterone and other salt-retaining hormones
17 K.S. :- 17-ketosteroids
A.D.H. :- pituitary vasopressin
11-oxy :- 11-oxysteroids

FIGURE LXVI

Postulated Causes of Diuresis in Patients Treated by Cortisone.



Vertical Axis = Pituitary Gland - Adrenal Cortex
D.C.A. :- aldosterone and other salt-retaining hormones
17 K.S. :- 17-ketosteroids
A.D.H. :- pituitary vasopressin
11-oxy :- 11-oxysteroids

very temporary amelioration of the abnormal osmotic pressure and, should diuresis occur, it will be temporary (since the basic fault persists) unless spontaneous remission is provoked.

It seemed that the mechanism of adrenal depression was purely an action on sodium and water metabolism independent of plasma proteins. That this action of cortisone does not depend on any specific effect on the proteinuria seemed clearly shown by the case of I.A.(C.1.). This boy, aged five years, suffered from gross oedema, the resultant of marked hypoproteinaemia accompanying coeliac disease. Both serum albumin and serum globulin were reduced but the plasma cholesterol level was normal. A course of cortisone, 200 mg. daily (with 2 grammes of potassium chloride) was given for five days and was followed by a sharp diuresis and the loss of 7 kilogrammes in weight(= 50 per cent. of his body weight). Oedema thereafter was minimal for some months, although the serum proteins were little altered and eventually the oedema disappeared. At no time was protein detected in the urine of this boy, precluding any action on proteinuria as a possible cause of diuresis. It is paradoxical to find in 1959 (Gordon) that such cases have in fact a leak of albumin into the gut and this may well have been improved by steroids!

The same general pattern was used as an explanation for diuresis following ACTH or cortisone therapy (Figures LXV and LXVI). During treatment with exogenous cortisone, depression of the pituitary and consequently of the adrenal cortex occurs. The withdrawal of such exogenous cortisone is followed by a period of days before pituitary and/

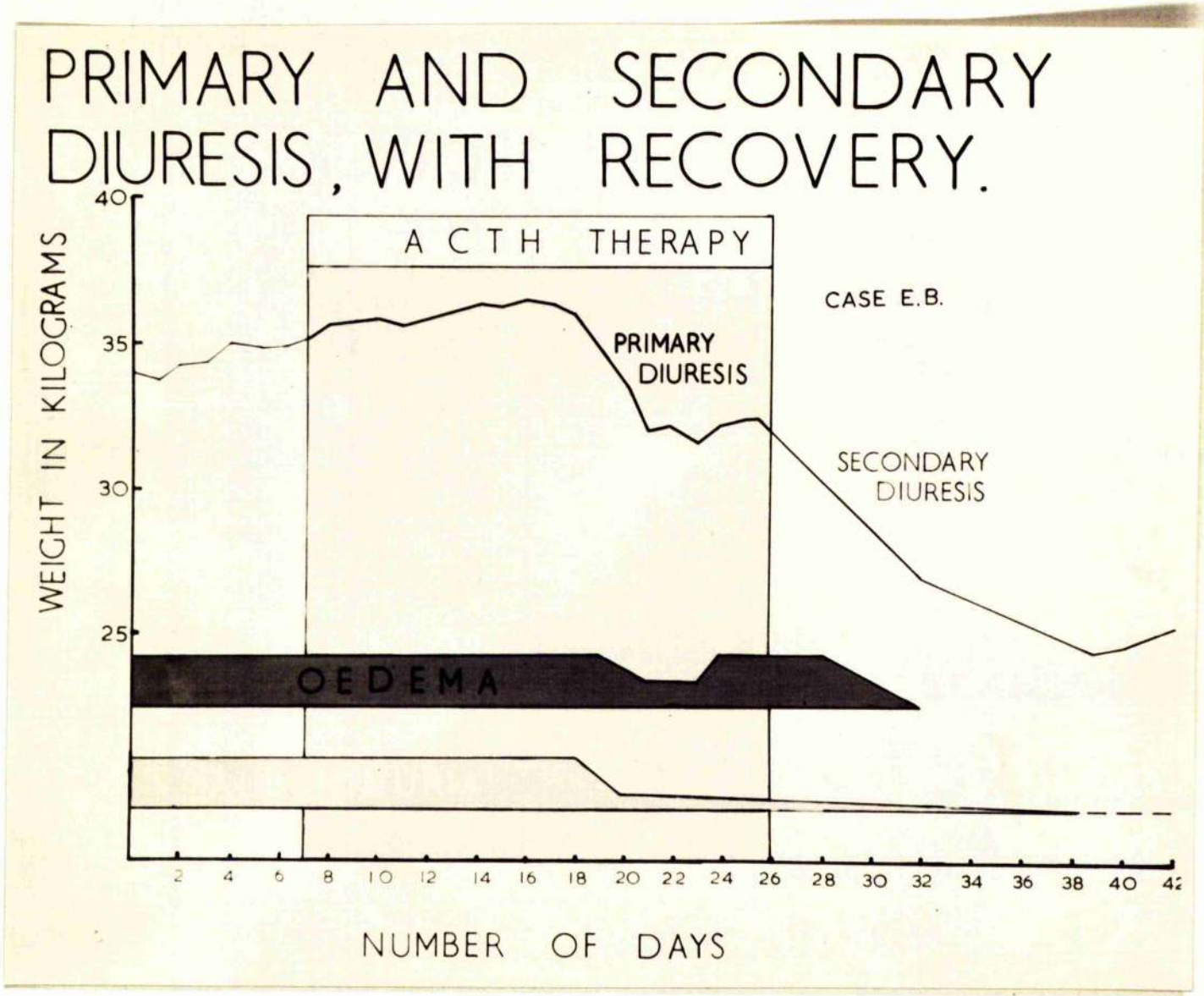
and adrenal function return to normal. When exogenous ACTH is given, and excess of endogenous cortisone results and depression of pituitary function with hyperactive adrenal cortices results. On stopping treatment a transient loss of adrenal hormone again occurs until pituitary function is restored.

Such explanations were satisfactory for diuresis occurring after treatment but did not account for water and salt loss during ACTH or cortisone therapy. Several explanations for this phenomenon have been advanced by Thorn et al. (1950). They suggested that renal "tubular fatigue" may result from a sustained high level of 11-oxysteroids or that these hormones may indulge in competitive inhibition with the desoxycorticosterone-like hormones, or that increased glomerular efficiency may occur during ACTH or cortisone therapy. None of these explanations were convincing. Two other possible theories suggested themselves. Firstly, if cortisone depressed the activity of the anterior pituitary gland, it might also depress the function of the posterior pituitary, provoking water diuresis and consequent sodium loss during treatment. This suggestion of primary water loss during treatment and sodium loss after treatment (diagramatically shown in Figs. LXV and LXVI and illustrated in relation to case records in Figs. LXVII and LXVIII) and the demonstration of an excess of antidiuretic activity in the blood of nephrotic patients (Lauson et al. 1952; Barlow, 1952) led to the research on pituitary peptides described earlier.

Secondly, the improvement following cessation of short courses/

FIGURE LXVII

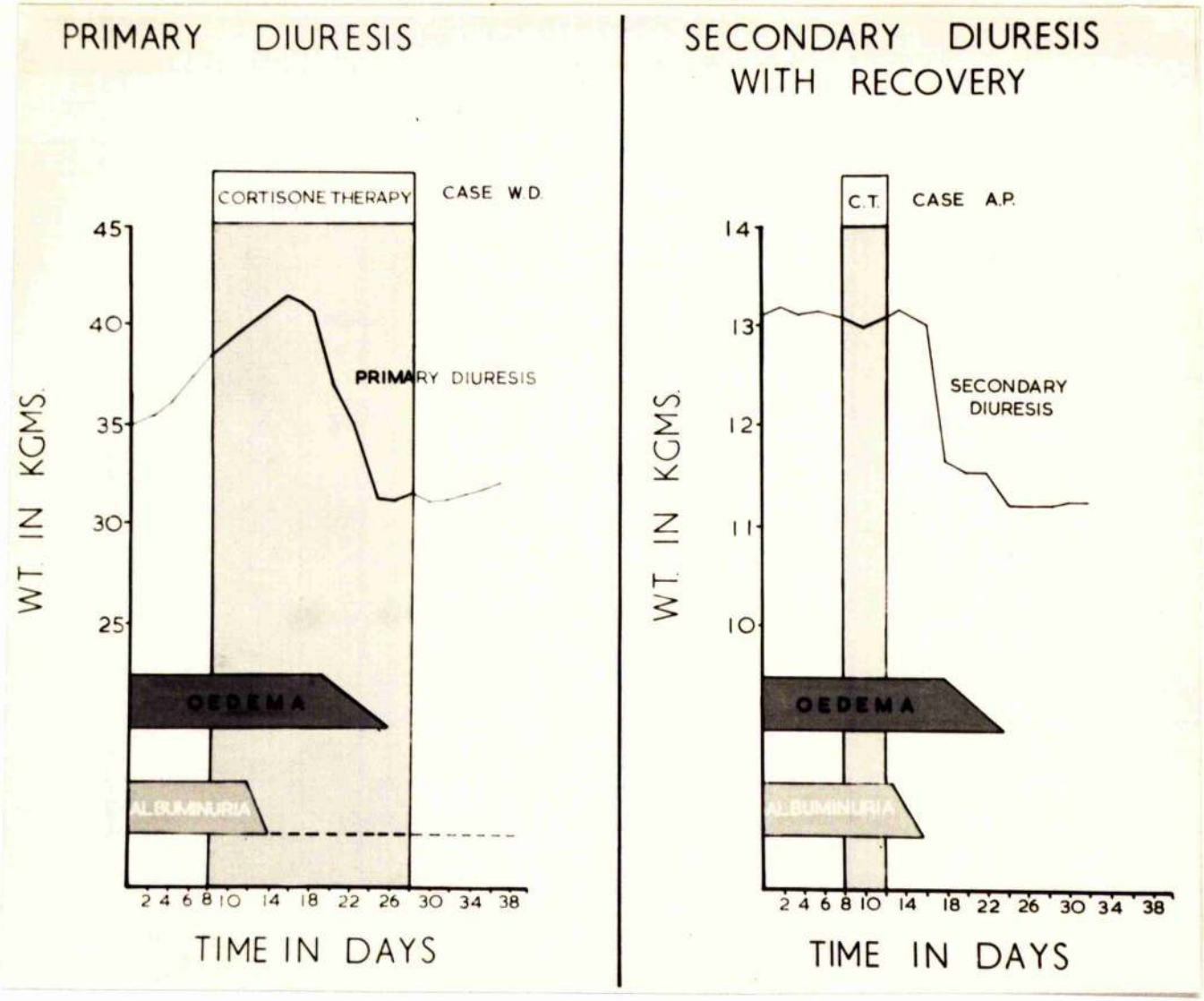
A.C.T.H. Therapy



The first diuresis is thought to be due to endogenous adrenal depression and the latter diuresis to cessation of sodium-retaining therapy.

FIGURE LXVIII

Cortisone Therapy.



In the first example diuresis occurred during prolonged therapy and in the latter example followed a brief course of cortisone.

courses of cortisone might in fact be related to the onset of treatment with the benefits disguised by the sodium retention during therapy, or delayed until more than five days from the start of therapy. This explanation would account for the apparent paradox of diuresis occurring both during and/or after hormone treatment. As a corollary to this idea a hormone with cortisone-like action, free of sodium retention, might produce improvement constantly during treatment. Such a hormone was sought in vain until September 1955 when Messrs. Pfizer Ltd. synthesized "Deltacortril" (Delta-1-hydrocortisone, now called prednisolone) claimed to have these properties (Bunim, 1955) and sent a supply for chemical trial (Arneil, 1956a & c, 1958).

Before proceeding to the later findings it is interesting to review the conclusions arrived at from these very confusing preliminary investigations. These were as follows:

(1) Both ACTH and cortisone at first increased proteinuria, which then decreased markedly in most cases apparently irrespective of:-
   (a) the continuation of treatment;
   (b) the occurrence of diuresis;
   (c) the level of serum proteins.

(2) Both ACTH and cortisone provoked diuresis in a number of instances and this could apparently be independent of:-
   (a) the continuation of treatment;
   (b) the diminution of proteinuria;
   (c) the level of serum proteins.

(3)/

(3) Both ACTH and cortisone tended to return the level of serum proteins and of each component fraction towards average levels.

(4) Both ACTH and cortisone reduced serum cholesterol levels when diuresis and diminution in proteinuria occurred.

(5) Despite (1) and (2) above, it could generally be said that diminution of proteinuria, diuresis, increase in serum protein level and fall in blood cholesterol level were approximately parallel.

(6) It seemed likely that incidental sodium retention due to steroidal activity was modifying the time of occurrence of diuresis.

PREDNISOLONE THERAPY

Preliminary Group

Patients were selected on the same criteria as before. The children were kept in bed and received a diet containing less than 2 g. sodium daily without additional potassium intake. Urinalysis and biochemical investigation were carried out by the same methods as before. In four cases a forty-day course of prednisolone (δ-1-hydrocortisone) was given (60 mg., 40 mg., 20 mg., and 10 mg. daily for ten days each). In the fifth case a five-day course of 60 mg. daily followed by sudden withdrawal of treatment was employed (the type of treatment which had been employed with cortisone). The five/

five patients were all male, ranged in age from 2½ - 8½ years, and had suffered from nephrosis for periods varying from 1 - 260 weeks.

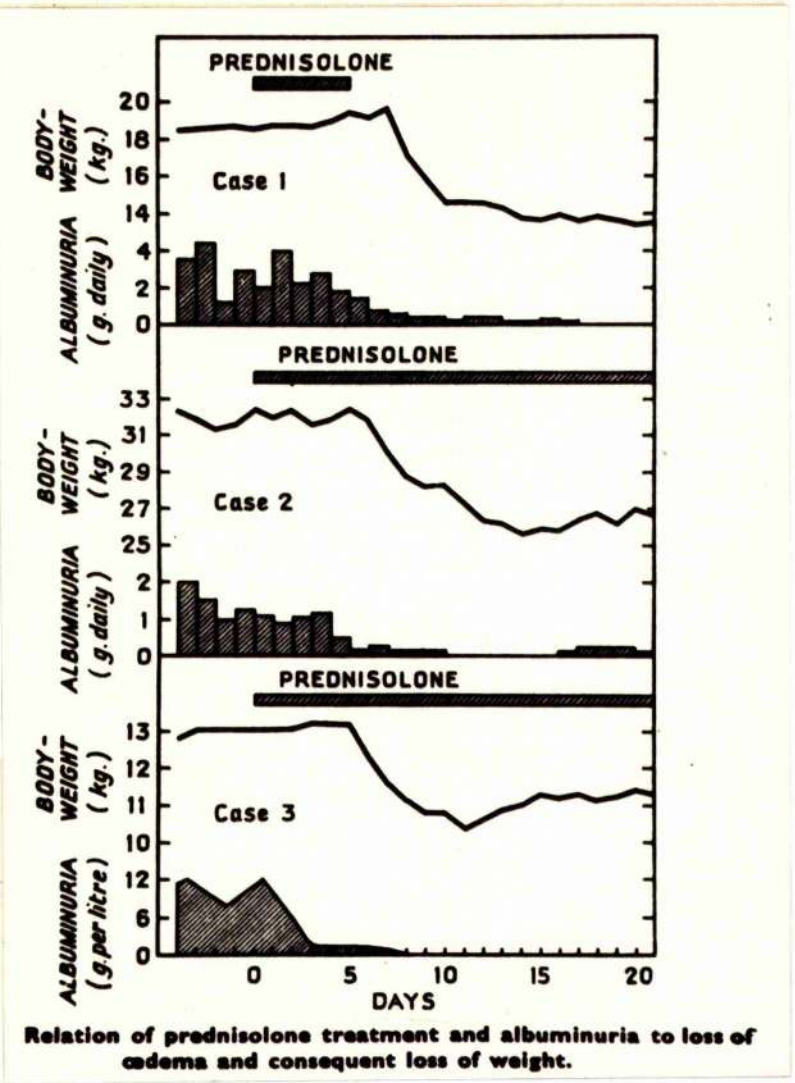
Effects of Prednisolone Treatment

In each case the proteinuria decreased markedly within six days of starting treatment. Prior to starting treatment the daily loss of protein in the urine had been 1 - 4 grammes; and this fell in each case to less than 0.25 g. per day. In four of the cases proteinuria disappeared completely in the next few days and is still absent 2 years later. In the fifth boy (who had suffered from gross oedema and proteinuria for more than five years) the proteinuria has greatly decreased but still amounts to about 250 mg. per day.

In all five cases diuresis occurred within eight days of initiating hormone therapy, and the patients have remained free of oedema. The periods range from 2 - 3 years. In Figure LXIX the relationship of diuresis to treatment, and to the decrease in proteinuria in the first three patients treated is shown; note that decrease in proteinuria appears to precede diuresis in each instance. These patients lost from 26 - 31 per cent. of their final body weight during diuresis and after this were seen to be gaunt and wasted. (Fig. LXX). Tissue anabolism rapidly followed and weight gain gradually occurred thereafter. (Fig. LXXI). In all five cases the serum protein level was initially low and rose to normal levels within forty days of treatment starting. Table XXX gives details of the biochemical findings in these five cases. It will be seen that the/

FIGURE LXIX

Relation of Diuresis (as judged by weight loss) and Albuminuria to Prednisolone Treatment in Three Cases.

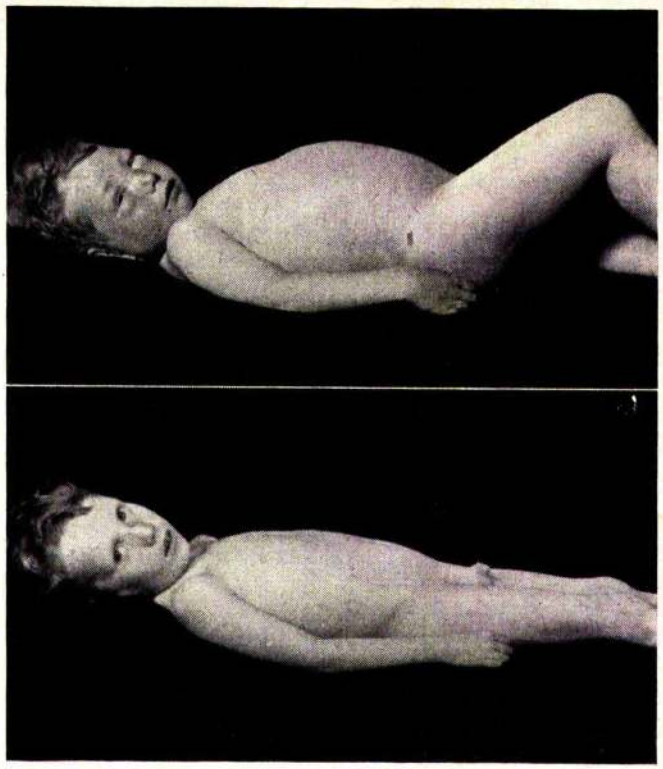


Relation of prednisolone treatment and albuminuria to loss of œdema and consequent loss of weight.

Relation of prednisolone treatment and albuminuria to loss of oedema and consequent weight loss.

FIGURE LXX

Prednisolone Induced Diuresis



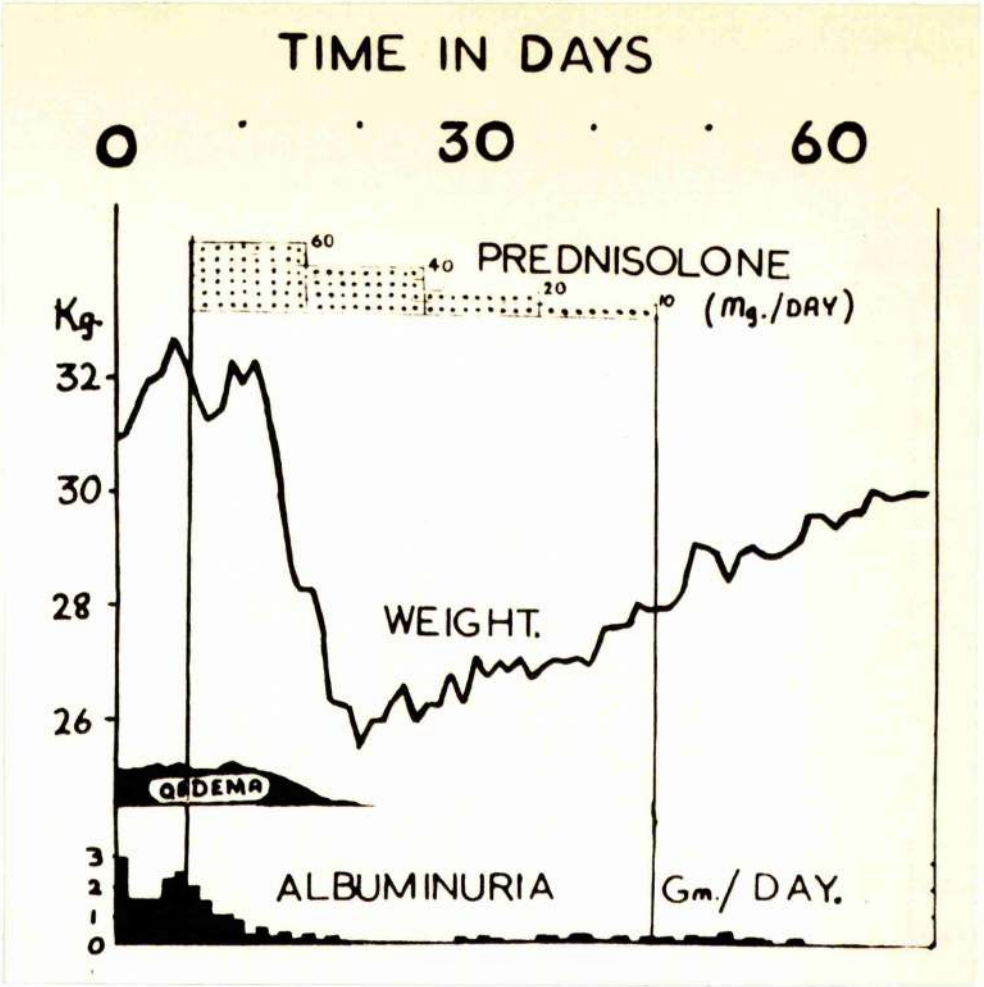
(a)

(b)

FIG. 1.—Two photographs of a child with nephrosis: (a) before steroid therapy; (b) three weeks later. Note how the rounded contours of œdema mask underlying wasting.

FIGURE LXXI

Diuresis Induced by Prednisolone



The initial weight loss is due to diuresis and is followed by a steady rise in weight from tissue anabolism.

TABLE XXX

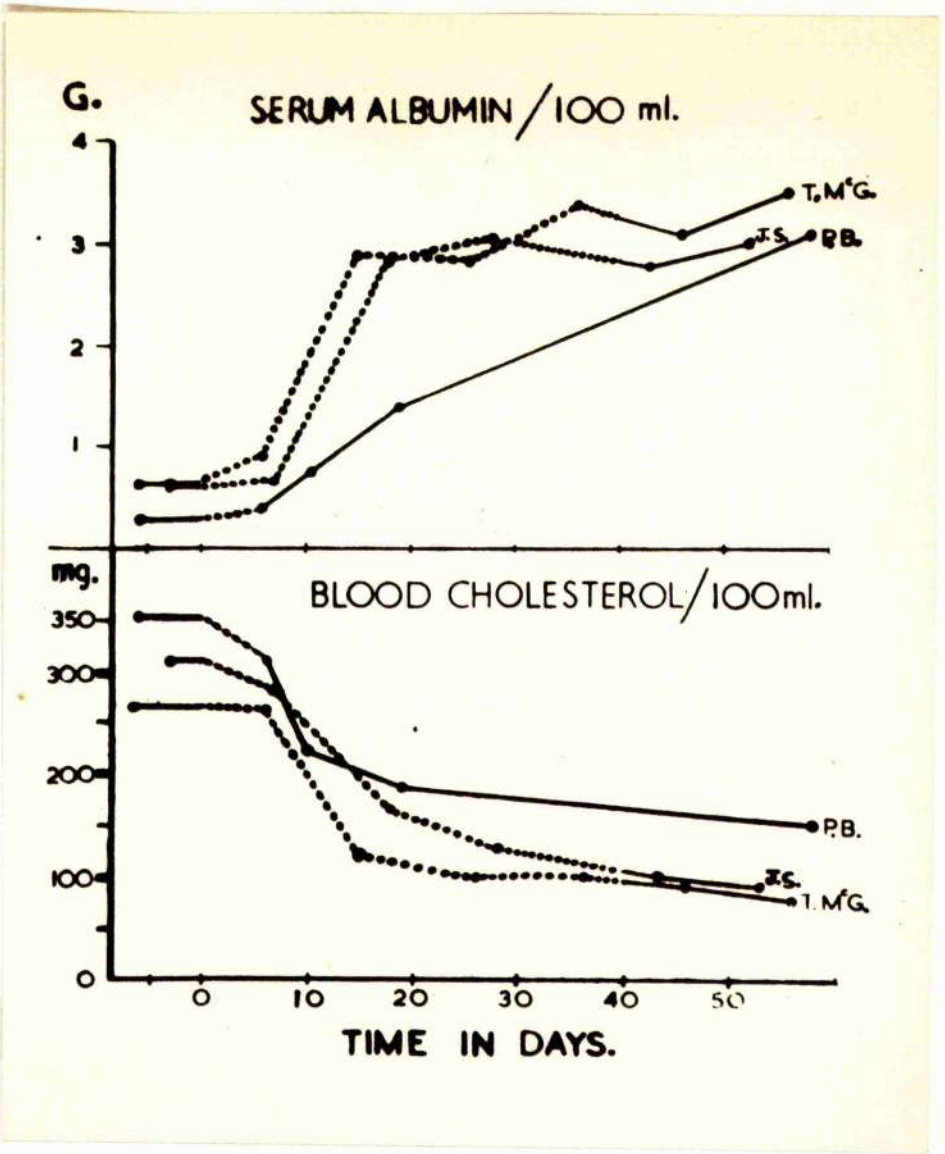
	Date	Serum Proteins G/100 ml.				Blood Cholesterol mg./100 ml.	E.S.R. Wintrobe mm/hr.
		Total	Albumin	Alpha & Beta Globulins	Gamma Globulins		
P.B.	28.9.55.	4.2	0.3	3.4	0.5	358	114
Treatment	9.10.55.	4.8	0.4	3.6	0.8	307	-
	13.10.55.	4.5	0.7	3.4	0.4	219	-
	21.10.55.	5.9	1.4	3.8	0.7	183	42
	22.11.55.	7.4	3.1	3.4	0.9	150	14
	3.1.56.	8.2	3.5	3.4	1.5	140	15
	6.3.56.	5.9	2.2	2.7	1.0	67	12
	30.4.56.	6.7	3.2	2.4	0.9	115	9
T.McG.	8.11.55.	4.8	0.6	3.4	0.8	265	46
	19.11.55.	6.0	0.9	4.1	1.0	260	-
	29.11.55.	6.2	2.9	2.1	1.2	120	14
Treatment	9.12.55.	7.2	2.8	2.8	1.6	100	16
	19.12.55.	7.4	3.4	2.0	2.0	100	10
	29.12.55.	7.4	3.1	2.7	1.6	95	9
	9.1.56.	7.4	3.5	2.3	1.2	80	12
	18.1.56.	6.9	2.5	3.2	1.2	90	9
	30.1.56.	6.1	2.7	2.2	1.2	91	7
	10.2.56.	5.0	2.3	2.1	0.6	43	5
	24.2.56.	5.4	2.4	2.3	0.8	100	7
	15.3.56.	5.3	1.9	2.3	1.1	67	12
	4.4.56.	5.5	2.5	1.8	1.2	51	11
	27.4.56.	5.2	2.5	2.0	0.7	86	12
J.S.	22.11.55.	5.0	0.6	4.0	0.4	310	116
	1.12.55.	5.7	0.6	4.1	1.0	280	43
Treatment	12.12.55.	6.8	2.9	3.3	0.6	165	15
	22.12.55.	6.9	3.0	2.7	1.2	127	4
	6.1.56.	7.1	2.8	3.0	1.3	100	2
	16.1.56.	7.1	3.0	3.2	0.9	86	27
	26.1.56.	6.7	3.1	2.3	1.3	103	6
	24.2.56.	5.6	2.7	2.1	0.8	105	8
	15.3.56.	6.6	2.6	3.0	1.0	190	9
Treatment	2.4.56.	7.4	2.8	2.6	2.0	115	7
	9.4.56.	8.1	4.0	3.0	1.1	156	5
	23.4.56.	6.4	3.3	2.2	0.9	86	12
D.S.	3.2.56.	4.8	-	-	-	467	-
	10.2.56.	4.5	0.7	3.3	0.5	230	44
	17.2.56.	6.4	-	-	-	176	-
Treatment	24.2.56.	5.7	2.6	2.4	0.7	90	16
	5.3.56.	6.8	3.0	2.8	1.0	76	11
	16.3.56.	6.6	3.8	2.0	0.8	91	1
J.R.	27.2.56.	4.5	0.7	3.3	0.5	247	49
	9.3.56.	4.8	1.0	3.2	0.5	167	22
Treatment	19.3.56.	6.1	3.9	1.8	0.4	105	11
	29.3.56.	7.1	2.8	3.6	0.7	111	10
	9.4.56.	6.8	3.5	3.4	0.9	75	5
	23.4.56.	6.7	3.9	2.3	0.5	52	10

the levels of serum albumin were initially very low but rose to within normal limits on prednisolone treatment in each case (Fig. LXXII). Although these improved levels were maintained the optimal biochemical findings were at the end of the forty day treatment period. It is intensely interesting to note that diuresis occurred with the level of serum albumin below 1.0 g. per 100 ml. in all five instances. The levels of gamma globulin which were initially low, rose in all cases, and have remained materially higher since then. The blood cholesterol levels which initially were above 260 mg. per 100 ml. fell rapidly to below 150 mg. in the like volume, and the erythrocyte sedimentation rates fell from very high levels to within normal limits in all five cases.

These findings are most interesting but even more important is the fact that the proteinuria of nephrosis was consistently reduced during the administration of prednisolone. No previous form of treatment has consistently caused such a change and it seems probable that other improvements such as the rise in serum albumin and gamma globulin, the fall in the blood cholesterol and the erythrocyte sedimentation rate and the eutrophy of the patient are sequential to the alteration in urinary protein loss. The mechanism involved remains obscure but prednisolone cannot act simply by blocking some noxious influence since improvement continues when treatment has stopped (as in the patient treated for five days). It seems that exhibition of the drug benefits the nephron, either directly or by upsetting some extrinsic factor (such as/

FIGURE LXXII

Relation of Serum Albumin and Blood Cholesterol Levels to Prednisolone Treatment.



Treatment periods indicated by interrupted lines.

as an abnormal antigen/antibody reaction) which is acting upon it, but that continuation of improvement is not necessarily dependent on continued medication.

Further Experience with δ-1-Hydrocortisone

Although the main group of cases numbering 168 stopped in early 1957 a number of cases have been treated with prednisolone since then and some notes on these may not be out of place here. Thirty children have been treated with prednisolone. Diuresis occurred in 25 of these, proteinuria lessened in 24, and 17 are asymptomatic. In dealing with such a chronic and unpredictable disease as nephrosis it would be foolish to place reliance on a small number of cases treated for such a short time. Nevertheless tabulation of immediate results of ACTH, cortisone and prednisolone treatment suggest that the last is significantly superior to the earlier hormones.

Condition of Oedematous Nephrotics Eight Weeks After Starting Hormone Therapy

	A ACTH	B Cortisone	C Prednisolone	D Prednisolone*
No. of cases	13	14	30	25
Free of oedema	3	2	25	24
Free of proteinuria	2	2	17	17

(* less "chronic" cases)

The figures given in column "C" are heavily biased against prednisolone because they include five nephrotic children whose illness had persisted for several years prior to treatment, despite previous courses of steroid and ACTH therapy. If these cases, which were really in a chronic/

chronic state, are excluded then results were as shown in colume "D". It will be seen that 96% become free of oedema and 68% free of proteinuria within 8 weeks. These immediate results of steroid therapy are incomparably better than those obtained with ACTH or cortisone. This may be because of larger doses and longer courses of treatment or because prednisolone has less sodium-retaining action and is more successful in reducing proteinuria. Be that as it may, one cannot escape the conclusion that prednisolone therapy is a significant advance in that it accelerates the disappearance of proteinuria, hypoproteinaemia, oedema and to a lesser extent, hypercholesterolaemia in nephrotic patients.

In an unpredictable disease of such a variable course as nephrosis it would be foolish to dogmatise on slender evidence. Whereas it was very unusual for a patient to relapse once proteinuria had entirely disappeared prior to the advent of steroid therapy, this is no longer true. It is now a common occurrence for a patient given intensive steroid therapy for a short period to lose all proteinuria, but relapse fairly frequently ensues when dosage is reduced too rapidly. When the cases which have been given prednisolone therapy and followed for two years are considered the results are as follows (the five "chronic" cases excluded).

State 2 Years After Onset/

State 2 Years After Onset

	Prednisolone Treatment	All Other Cases
Dead	2 (11%)	42 (29%)
Proteinuric	4 (21%)	46 (32%)
Asymptomatic	13 (68%)	57 (39%)

It will be seen that not only is the mortality significantly lower but that the persistence of residual proteinuric is less frequent. Since approximately 50% of children with proteinuria persisting two years after onset subsequently become asymptomatic it seems highly probable that the recovery rate for the prednisolone group will be in the neighbourhood of 80%.

These facts are more readily appreciated in histographic form and in Figure LXIII the situation is clearly summarised. The various treatment groups are as follows:

Group A: 1929-36 Pre-sulphonamide group.

Group B: 1937-45 Sulphonamide available for treatment of infection.

Group C: 1946-50 Penicillin, chloramphenicol and streptomycin available for treatment of infection.

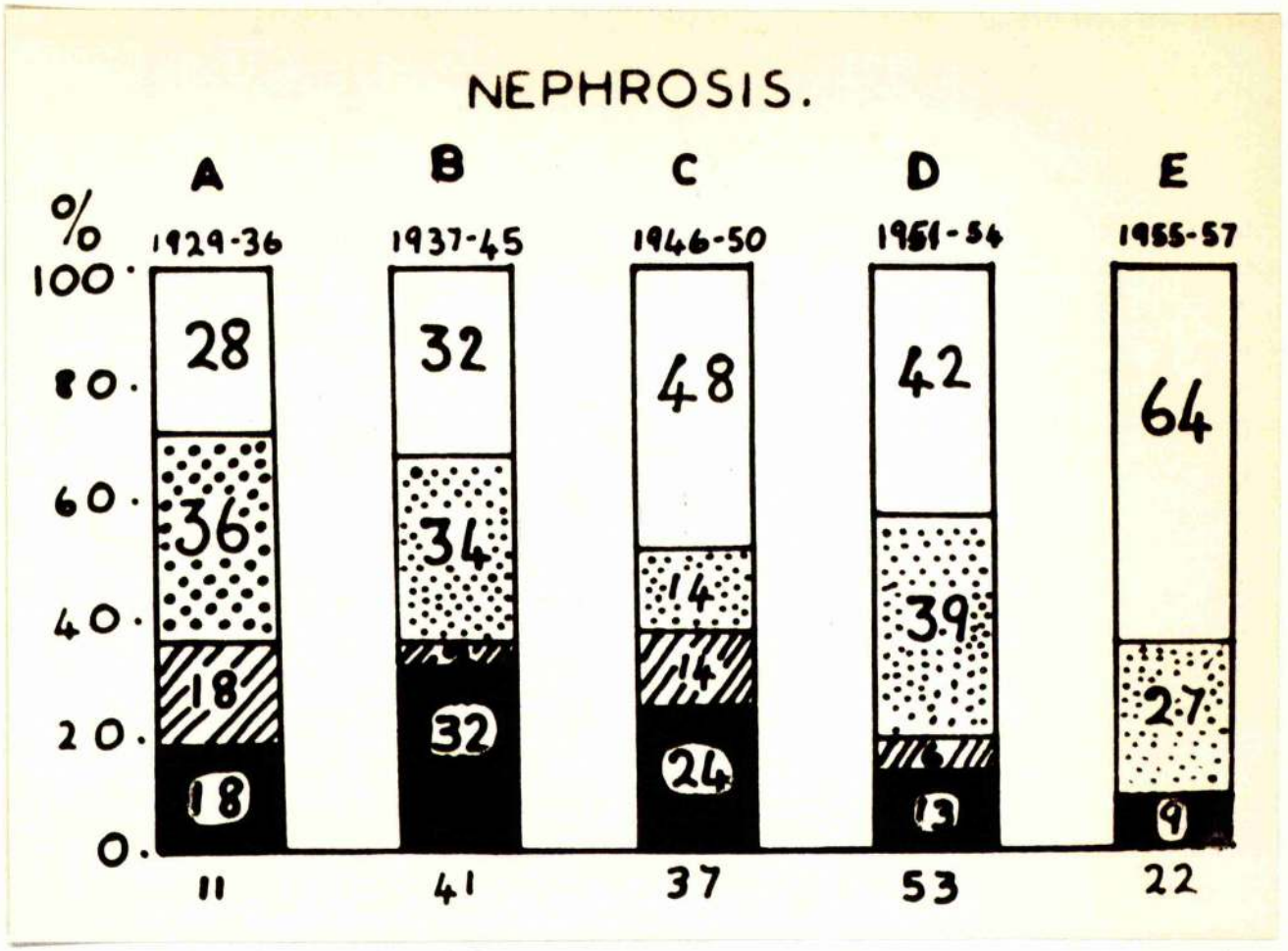
Group D: 1951-54 Cortisone and ACTH short-term therapy available.

Group E: 1955-57 Prednisolone therapy in large doses available.

All these patients have been followed for at least two years and the clinical state referred to in each patient is two years from onset. The columns are therefore comparable. It is obvious that a very significant improvement in outlook has followed the use of prednisolone/

FIGURE LXXIII

Clinical Status Two Years After Onset.



The dark areas represent deaths due to infection, the striped areas deaths from renal failure; the stippled areas continuing proteinuria, and the blank areas the asymptomatic state. All figures expressed as percentages except those beneath the abscissa which represent the number in each group.

prednisolone therapy. From the discussion in past pages it is clear that the deaths from infection have biased the figures against the pre-antibiotic era. Figure LXXIV corrects this bias by excluding deaths from infection. It is at once clear that quite apart from infection a decrease in deaths and in the frequency of persisting proteinuria has occurred. The higher death rate during the period 1946-50 is ascribed to the use of mercurial diuretics.

OTHER FORMS OF STEROID THERAPY

Any new method of treating nephrosis must be measured against the improved rate of recovery and the beneficial effects of prednisolone therapy. For a new substance to be of value it would require to be either a more active steroid than prednisolone with fewer side effects or a substance effective in removing proteinuria or oedema in patients in whom prednisolone therapy had proved ineffective. It is against this background that three new substances, triamcinolone, methylprednisolone and dexamethasone are assessed.

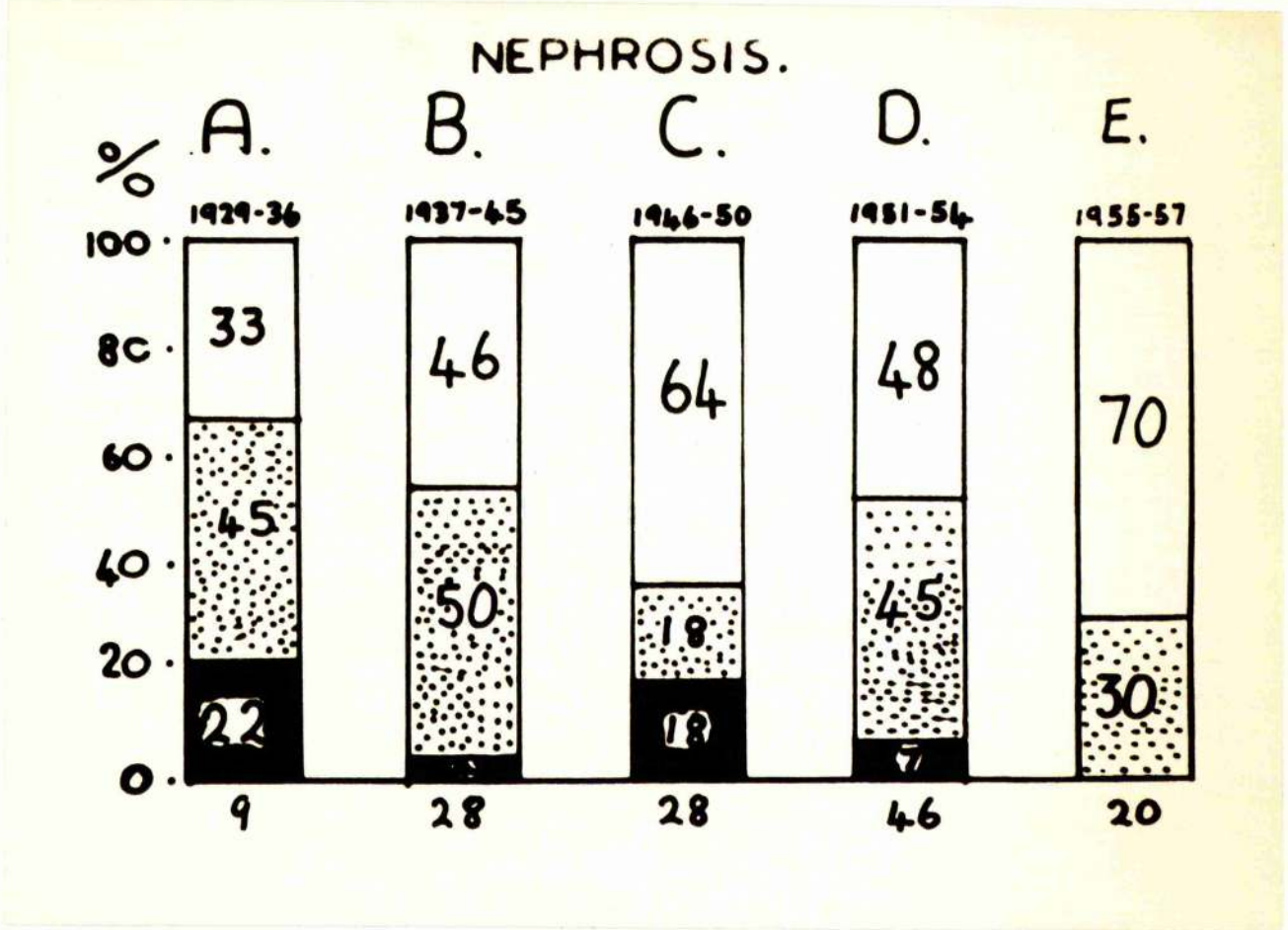
Triamcinolone (9-α-fluoro-16-α-hydroxy prednisolone)

This synthetic steroid with fluorine at the 9-alpha position was claimed to have greatly increased gluco-corticoid activity whilst the hydroxyl radical at the 16-alpha position reduced sodium and water retention. It thus seemed to offer greater glucocorticoid activity than prednisolone at a lower dosage level and with fewer side effects.

The/

FIGURE LXXIV

Clinical Status Two Years After Onset
(Less deaths from infection)



The dark areas represent deaths from renal failure, the stippled area persisting proteinuria and the black area the asymptomatic state. All figures refer to percentage except those below the abscissa which refer to total number in each group.

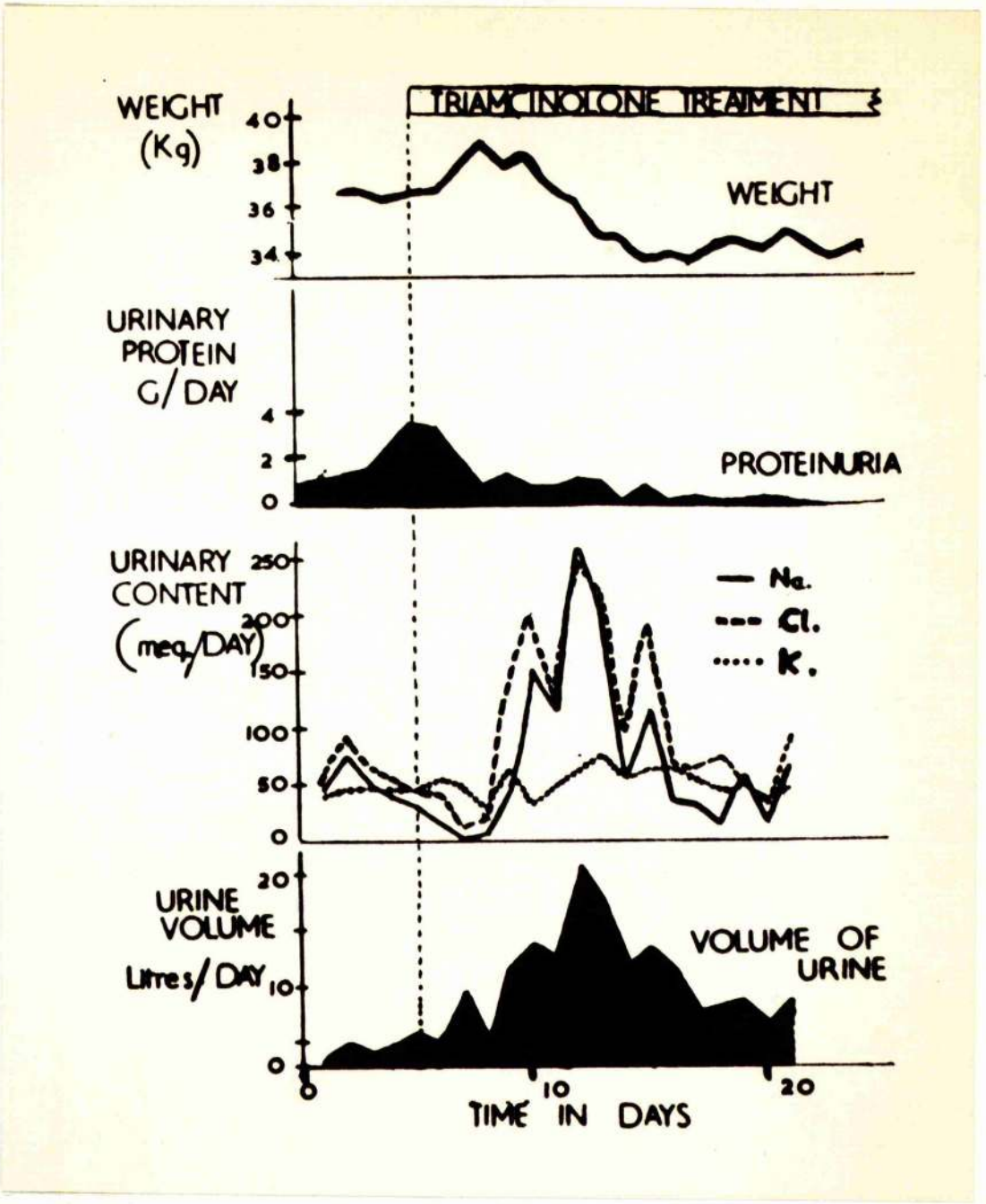
The dosage of triamcinolone recommended for the nephrotic syndrome is 20 mg. daily by mouth for a period of 30-45 days (Hellman et al. 1957). On this dosage schedule results were not satisfactory and consequently the amount given initially was increased to 40 mg. daily. At this level of dosage the beneficial effects of the steroid were at once apparent. The results obtained in treating one case successfully are shown in Figure LXXV.

Seven patients with nephrosis have now been given treatment with triamcinolone. Of these cases, five have responded with satisfactory diuresis and in four proteinuria ceased or has been reduced to minimal amounts. The serum proteins and cholesterol levels have been returned to within normal limits and the erythrocyte sedimentation rate has returned to normal. The dosage of triamcinolone given orally was initially 40 mg. daily and this level was maintained until diuresis and decrease in proteinuria commenced or for 20 days. The level of daily dosage was then reduced to 32 mg. and, if the case continued to improve, was gradually reduced to 12 mg. daily after approximately six weeks treatment. This level of dosage was then continued for a period of 3 - 6 months provided that proteinuria remained minimal or absent and the hormone was then stopped. If gross proteinuria recurred during reduction of hormone intake or following the end of treatment then the amount of triamcinolone being given was stepped up until control of proteinuria was obtained.

Side-effects Patients receiving triamcinolone became moon-faced as/

FIGURE LXXV

Triamcinolone Induced Diuresis



Note the close parallelism between daily loss of water, sodium and chloride in the urine.

as when other steroids are employed. All become obese but in two anorexia and lethargy was later noted and weight was lost. One patient developed marked hypothermia which responded rapidly to symptomatic treatment. Five children developed widespread hirsutism and another was noted to have faecal impaction. Collapse of vertebral bodies have also been noted. These side-effects are much more frequent than those produced by prednisolone treatment.

It may be that triamcinolone, weight for weight, is slightly more active than prednisolone but the high incidence of side-effects seems to outweigh any advantages. Its use has been discontinued.

Methylprednisolone (6-methyl-prednisolone)

This was used in one patient who rapidly became asymptomatic. It does not seem to differ significantly in action from prednisolone.

Dexamethasone (9-α-fluoro-16-α-methyl prednisolone)

This steroid was used very successfully on two cases of which one was a severe nephrotic who had relapsed after three insufficient courses of prednisolone. It has rendered him asymptomatic. The dosage employed was 10 mg. daily initially tapered down over a period of four months. This was given with a restricted caloric diet containing less than 88 milli-equivalents of sodium daily.

The second case was a girl with straightforward nephrosis of one month's duration. She responded rapidly to treatment with dexamethasone and a very accurate assessment of her sodium, potassium, chloride, protein and water output in urine for each

six/

six hour period during a period of three weeks duration was made.

The results obtained in treating these two cases with dexamethasone are graphically shown on Figures LXXVI and LXXVII.

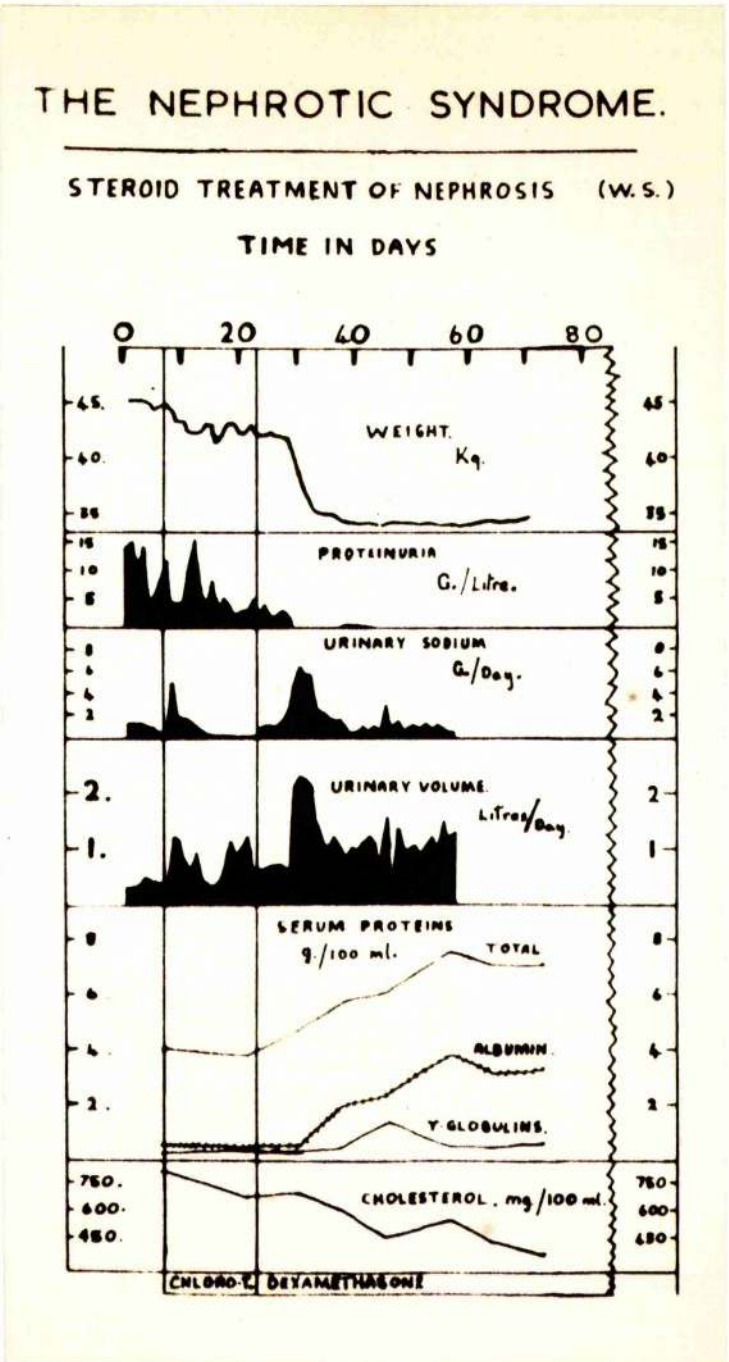
This steroid quite plainly requires a much lower dosage level than prednisolone but since it is at present more expensive this advantage is largely outweighed. Considerable further investigation is required.

Chlorothiazide Therapy

Chlorothiazide (6-chloro-7-sulphanyl-1,2,4-benzothiadiazine-1, 1-dioxide) is not a steroid but the structure of this substance is interesting in that it contains not merely an unusual fused ring system wherein is sulphur in its highest oxidation state but also a sulphanyl group attached to a benzenoid ring. Chlorothiazide influences the handling of electrolytes by the renal tubules, causing a marked increase in the elimination of sodium, potassium, chloride, and (at high dose levels) bicarbonate in the urine of experimental animals. Natriuresis is accompanied by water diuresis. Chlorothiazide may be given orally, or intravenously, and has a comparatively short period of activity (4 - 6 hours). The substance antagonises the sodium retaining activity of such steroids as 9-alpha fluoro-hydrocortisone and cortisone itself.

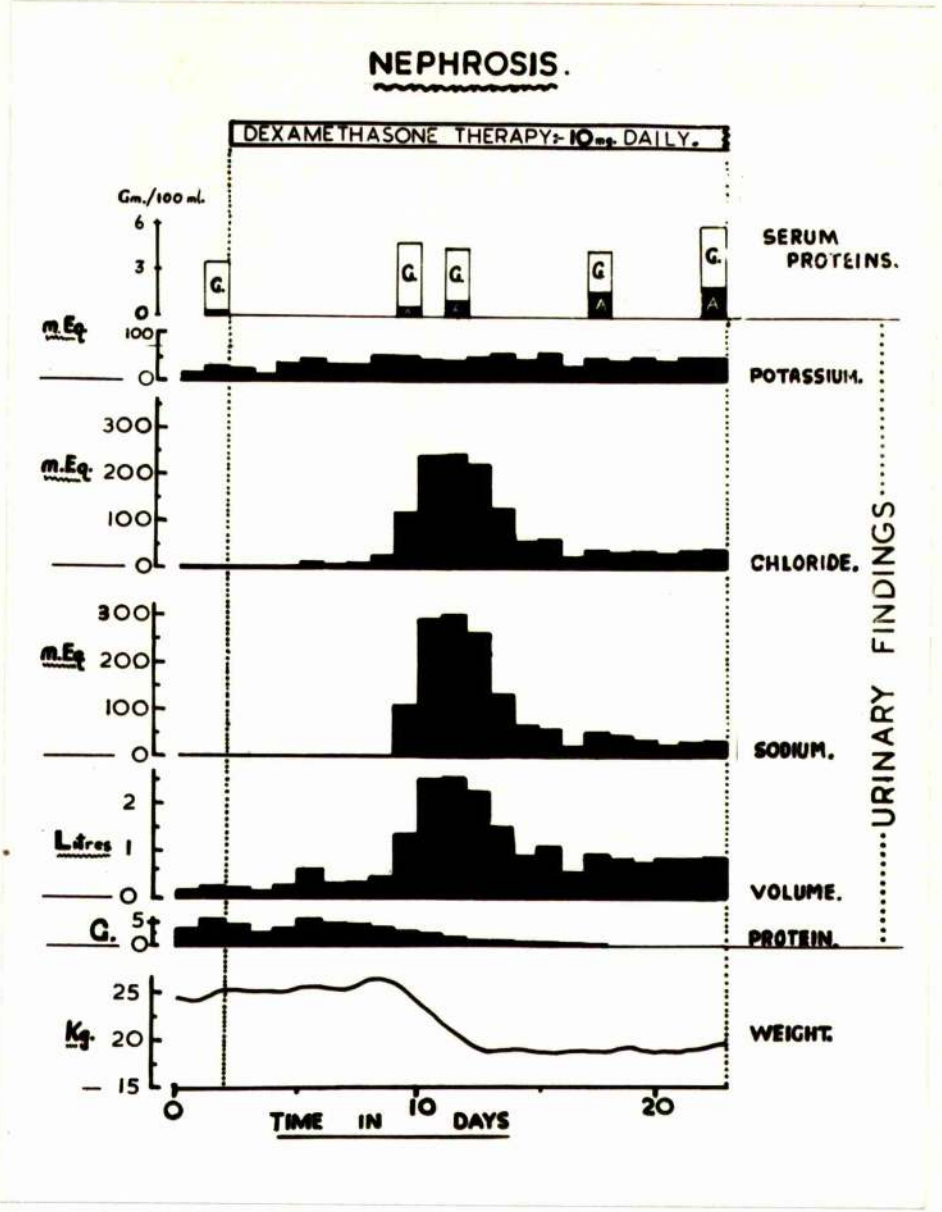
In view of the experimental results obtained with this substance it was decided to test the efficacy of the agent in freeing nephrotic patients of oedema. With this point in mind a nephrotic who had been oedematous/

FIGURE LXXVI



Note the coincidence of diuresis and reduction in proteinuria. The serum protein levels ameliorate much more slowly.

FIGURE LXXVII



The effects of dexamethasone on the serum protein levels and the daily electrolyte content of the urine are shown. Note the decline in proteinuria and the loss in weight as diuresis occurs.

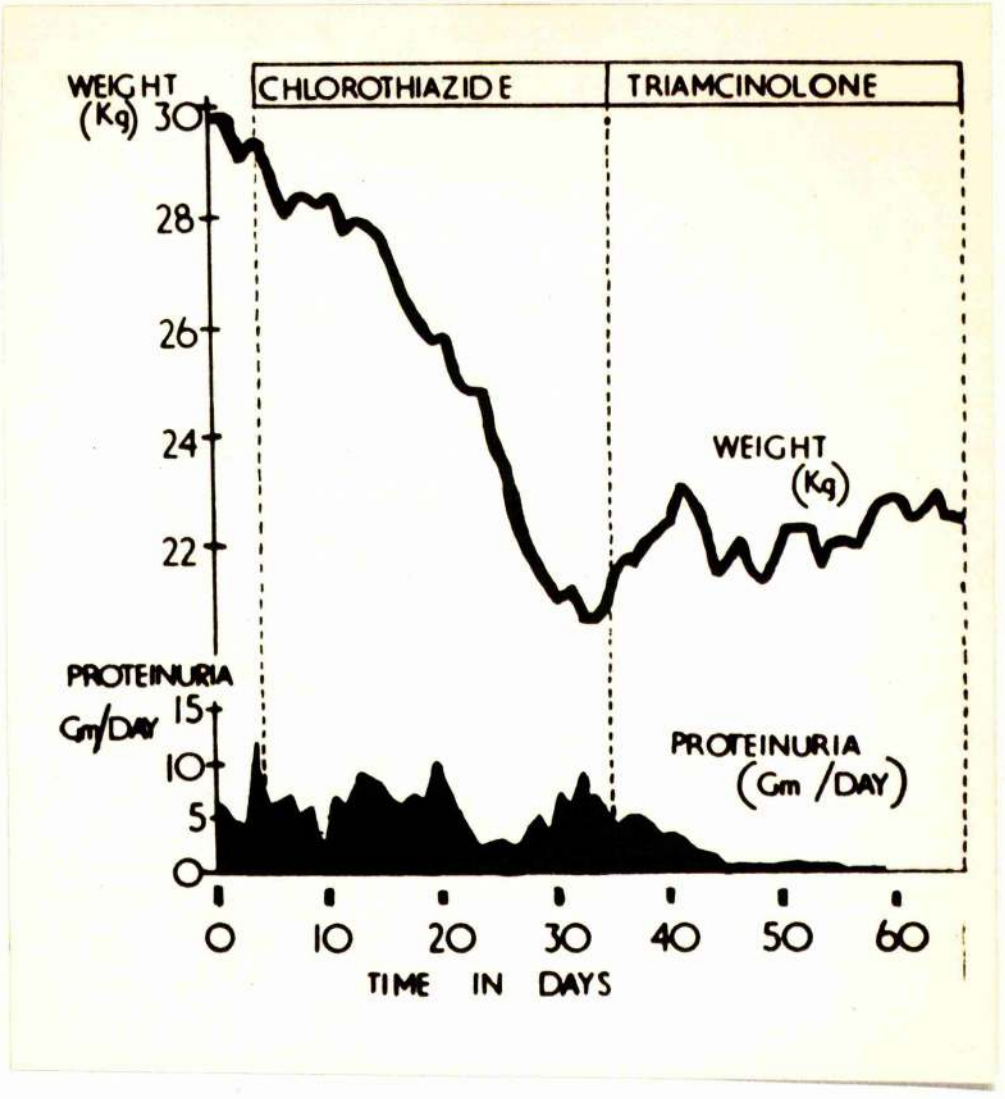
oedematous for some months was treated with chlorothiazide (Fig. LXXVIII). Diuresis of water, sodium and chloride began, within twelve hours and a dramatic fall in weight occurred as the boy became free of his ascites and oedema. Despite this fact there was virtually no improvement in the daily loss of protein in the urine. After 21 days of treatment chlorothiazide was discontinued and triamcinolone therapy begun. He immediately began to gain weight and this presumed retention of fluid lasted for seven days and then levelled off. Of greater significance was the fact that within ten days of starting steroid therapy his proteinuria had decreased markedly and six months later was scarcely perceptible. It seemed therefore that in this boy chlorothiazide caused a diuresis, but did not decrease proteinuria although such a reduction was readily effected with steroid therapy.

It was then decided to test the effect of chlorothiazide on the oedema of nephrotics who had repeatedly failed to respond to steroid therapy. The effects on one such child are shown in Figure LXXIX. It will be seen that diuresis of water, sodium and chloride began shortly after the start of chlorothiazide therapy and that loss of weight was considerable as the oedema was shed. The daily loss of protein in the urine decreased but not to a significant extent. From this result it seems that chlorothiazide will induce loss of water, sodium and potassium in a patient with nephrotic oedema who has failed to respond to steroid therapy. These observations have subsequently been confirmed in a number of patients. It should

be/

FIGURE LXXVIII

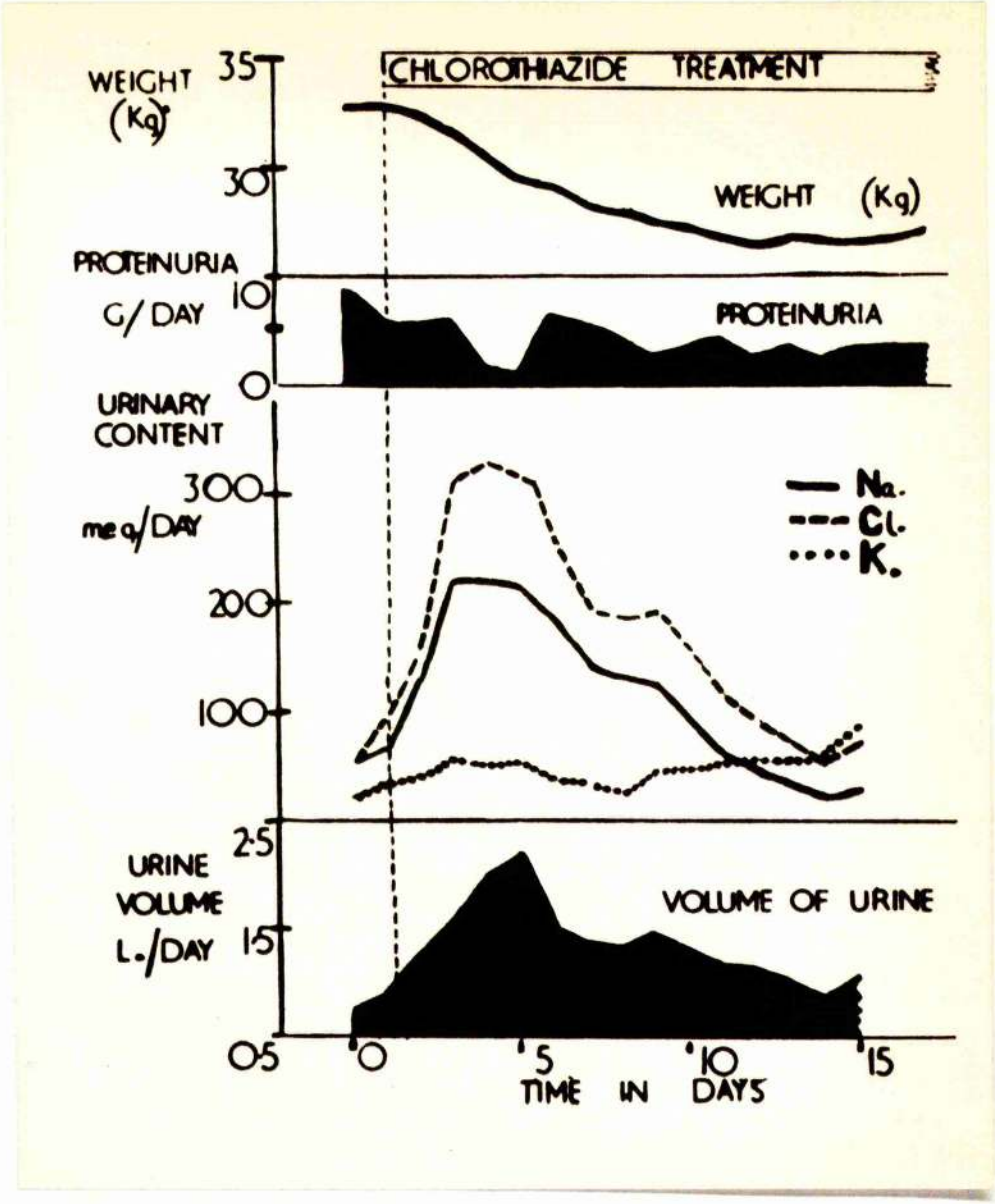
Comparison of Effects of Chlorothiazide and Triamcinolone on Nephrosis



Note that chlorothiazide caused diuresis but not significant decrease in proteinuria.

FIGURE LXXIX

Chlorothiazide Induced Diuresis



Note the close parallelism between the daily loss of water, sodium and chloride and the persistence of proteinuria.

be noticed that the potassium lost in the urine increased as the treatment was continued and in order to avoid the risk of hypokalaemia it is prudent to give all such patients 1 - 2 g. of potassium daily (i.e. 2 - 4 g. of potassium chloride daily).

Neonatal nephrosis is a congenital form of the disease which is usually fatal within a few months of birth. Steroid treatment has been of little or no value in treating these cases and our experience in four cases here has confirmed this finding. Since these infants tend to remain in an oedematous state and are very susceptible to enteral infection it seemed that chlorothiazide therapy might prove of benefit. A female child aged 5 months who had been oedematous from birth, due to congenital nephrosis, and in whom steroid therapy had proved valueless was given 500 mg. chlorothiazide daily and this relieved the oedema. This dosage of chlorothiazide maintained her free of oedema for many weeks thereafter although the albuminuria persisted.

From these findings it would seem that chlorothiazide has the following effects in the treatment of nephrosis:

(1) Chlorothiazide in sufficient dosage causes diuresis of water, sodium and chloride, and to a lesser extent of potassium. The necessary dose varies from patient to patient. This effectively relieves oedema.

(2) Chlorothiazide treatment does not seem to reduce the amount of proteinuria significantly.

(3)/

(3) Chlorothiazide may remove the oedema of a patient refractory to steroid therapy.

(4) Chlorothiazide may remove the oedema of a patient with congenital nephrosis and may keep him oedema-free if adequate maintenance dosage is given for a period.

Final Remarks on Therapy

The investigations carried out so far have made it quite clear that adequate steroid therapy has the following beneficial effects.

1. Proteinuria decreases during treatment, in the large majority of cases, and often ceases.
2. Diuresis of water, sodium and chloride occurs.
3. Oedema and ascites disappear.
4. The levels of serum albumin and of gamma globulin rise.
5. The serum cholesterol content falls.
6. The nutrition of the patient improves.
7. The liability to infection of the patient decreases.
8. The erythrocyte sedimentation rate returns to within normal limits.

These changes may be of a temporary or a permanent nature. Individual patients recover after a short period of steroid therapy, others require long continued or repeated courses of treatment, and still others fail to respond to adequate and prolonged therapy. The response is more favourable in patients without initial haematuria and/

and when the disease has been present for less than a year.

Steroid therapy in our hands has produced the following side-effects:

1. "Moon-face" development in almost all children. This recedes when treatment stops, and is usually absent after one year.
2. Obesity of a Cushingoid type: this can be minimised by prescribing a limited caloric and carbohydrate intake throughout treatment.
3. Temporary glycosuria.

None of these side-effects has caused any serious alarm to the clinician or upset to the patient.

The toxic effects which we have observed in steroid therapy of nephrosis have been few and far between. The status epilepticus related to hypokalaemic alkalosis produced by ACTH in one boy has already been described. Faecal impaction apparently due to intestinal hypotonia occurred on five occasions, presenting as sub-acute intestinal obstruction. This readily resolved when an enema was given. When triamcinolone was used in lieu of prednisolone hirsutism was observed in most patients, muscle weakness in some patients and collapse of vertebral bodies and skeletal decalcification in one case. These apart, no serious toxic effects have been noted. Together with investigations here, parallel work in other parts of the world led to various types of treatment being devised. In the U.S.A. much enthusiasm for ACTH by injection was voiced until 1956, when prednisolone and prednisone came into use. The form of treatment advocated/

advocated there was to continue steroid therapy for many months, often in intermittent dosage. The idea of giving such treatment on perhaps only three days a week, was to stimulate endogenous adrenal activity. American nephrologists almost all gave continuous antibiotic therapy during treatment with steroids. Here this was found to be quite unnecessary as a routine, although sometimes used in individuals in whom repeated respiratory infections seemed to be triggering off relapses.

Finally, the scheme for optimal steroid treatment of nephrosis in childhood used here in July 1959 is appended.

A Dietetic Treatment

1. The diet to contain less than 88 m.eq. (2 g.) sodium daily during steroid therapy.
2. The diet during steroid therapy to be isocaloric with intake prior to treatment and low in carbohydrate content.

B Antibiotic Treatment

1. Any obvious infection to be treated by large doses of antibiotics, prior to steroid therapy.
2. Any infections occurring during therapy to be treated at once with appropriate antibiotic.
3. When recurring infections trigger relapse prophylactic antibiotic treatment to be given thereafter.

C Steroid Treatment

1. Prednisolone is the steroid of choice.

2./

2. The initial dosage 60 - 80 mg. per day, irrespective of the size of the patient.
3. The dosage to be reduced by 10 mg. every 10 days and discontinued provided proteinuria becomes and remains minimal ($<$100 mg./day) or ceases.
4. Should a return of gross proteinuria occur during diminution of steroid intake then dosage should be increased, and thereafter reduced more slowly.

D Chlorothiazide Treatment

This palliative treatment is aimed at reducing oedema when steroid therapy fails. Dosage of approximately 1 - 4 g. daily is required and 2 - 4 g. of supplementary potassium chloride should be given daily.

Results obtained in this investigation suggest that with this plan of treatment it is probable that a continuing asymptomatic state will be achieved in 80 - 85% of children with nephrosis. Since this represents a twofold increase on the number obtaining prior to 1951 (when this investigation began) these figures are encouraging, but many problems remain unsolved in the pathogenesis and treatment of nephrosis.

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VASOPRESSIC FACTORS AND THE DEVELOPMENT OF STEROID THERAPY IN NEPHROSIS

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The nephrotic syndrome which is characterised by capriciousness of course and unpredictability of outcome has long presented a clinical enigma and a therapeutic problem. Bi-directional partition chromatography of urine revealed a peptide excreted by patients with this disease and the like technique showed similar peptides to be present in posterior pituitary extracts. This proved to be the active polypeptide fraction of the latter. Attempts to measure the presence of excessive vasopressic activity in plasma of patients led to the use of a very delicate method of biological assay for vasopressin. This procedure was adapted to provide a new method of bio-assay for angiotonin more delicate than those previously available. This investigation led obliquely to the finding of unexplained pressor activity in plasma from hypertensive nephritics, in incubated plasma from normal subjects, and in blood bank plasma.

An endeavour was made to establish the clinical pattern and natural history of nephrosis in children. To this end 164 cases who had attended The Royal Hospital for Sick Children, Glasgow, between 1929 and 1957 were traced to 1959 or until death. Much/

Much information concerning the natural history of the syndrome was obtained. A number of factors which modified the course and prognosis of the disease were defined and a background against which to assess the value of new forms of therapy was established.

In 1951 cortisone and corticotrophin became available for clinical trial. The actions, side-effects, and toxic effects of these hormones were virtually unknown at this time. Initially short and rather tentative courses of such steroids were given to these nephrotic patients and the effects of treatment on oedema, ascites, proteinuria, serum proteins and electrolytes observed. Conflicting and puzzling results were obtained initially but clarification became possible when δ-1-hydrocortisone (which possessed much greater glucocorticoid activity relative to sodium retaining power) became available. Since 1956 a series of experimental therapeutic tests with prednisolone, triamcinolone, and dexamethasone has led to the evolution of effective therapy for nephrosis. Steroid therapy is used in judicious combination with sodium restriction, antibiotic therapy, and with chlorothiazide. This type of treatment is now widely employed and the significant improvement in the morbidity and mortality of nephrosis which has occurred in recent years may be consequential.

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