to produce intracellular oedema: this should be minimal when a twin-coil ultrafiltration artificial kidney (Aoyama and Kolff, 1957) is used, because this machine can effectively remove water at a rate of 800 ml./hour. Animal experiments (Alexander *et al.*, 1961) have shown that intravenous infusions of urea, similar to those used to decrease brain bulk at craniotomy (Stubbs and Pennybacker, 1960), produce a significant transitory increase of plasma volume which is believed to reflect withdrawal of fluid from body tissues as a result of a generalized blood-tissue osmotic gradient. To postulate that reversal of such a process in uraemic patients treated by haemodialysis, especially when a high rate of urea clearance is achieved and the plasma protein concentration is low, could produce a reversed osmotic gradient should therefore cause little surprise. Indeed, the development of cerebral oedema, which has been observed on occasion during haemodialysis, together with the gradual restitution of the neurological state which begins shortly after haemodialysis is stopped, can be reasonably attributed to urea-concentration differences in the intracellular and extracellular compartments and their subsequent spontaneous equilibration. The cause of the delayed urea diffusion remains obscure: it is possible that a specific non-diffusible urea moiety exists in such circumstances (Blackmore and Elder, 1961), but expansion of the extracellular compartment physically affecting urea transfer might also be significant.

Summary

Intracellular concentrations of urea were determined in muscle biopsies taken from 19 unselected patients with acute renal failure immediately after haemodialysis on a rotating-coil artificial kidney.

The values were compared with corresponding plasma urea values of venous blood samples withdrawn at the time of the biopsies and were found to be significantly greater in 14 instances and less in 5.

Post-dialysis hourly rates of rise of plasma urea concentration were determined in 14 unselected patients and the average time taken for equilibration with the respective pre-dialysis hourly rates of rise was found to be 15 hours.

The observations suggest that haemodialysis on a rotating-coil artificial kidney may sometimes, as a result of delayed diffusion of urea across cell membranes, be followed by an intracellular/extracellular urea concentration ratio greater than unity.

A raised intracellular/extracellular urea concentration ratio, by producing an osmotic gradient, could rationally explain the development of cerebral oedema during haemodialysis, its gradual spontaneous regression when haemodialysis is stopped, and the well-recognized temporary accelerated rate of rise of the plasma urea concentration during the first post-dialysis day.

We are indebted to Dr. Ruth Haslam and Dr. I. D. P. Wootton, of the Department of Biochemistry at the Postgraduate Medical School, for their technical assistance and critical interest.

REFERENCES

Alexander, S., Eaton, J. C., and Freedman, H. J. (1961). *J. Neurol., Neurosurg., Psychiat.*, **24**, 148.
Aoyama, S., and Kolff, W. J. (1957). *Amer. J. Med.*, **23**, 565.
Barnes, B. A., Gordon, E. B., and Cope, O. (1957). *J. clin. Invest.*, **36**, 1239.
Blackmore, D. J., and Elder, W. J. (1961). *J. clin. Path.*, **14**, 455.
Flear, C. T. G., Crampton, R. F., and Matthews, D. M. (1960). *Clin. Sci.*, **19**, 483.
Hastings, A. B., and Eichelberger, L. (1937). *J. biol. Chem.*, **117**, 73.
Loughridge, L. W., Milne, M. D., Shackman, R., and Wootton, I. D. P. (1960). *Lancet*, **1**, 351.
McCance, R. A., and Widdowson, E. M. (1951). *Proc. roy. Soc. (B)*, **138**, 115.
Murdaugh, H. V., and Doyle, E. M. (1961). *J. Lab. clin. Med.*, **57**, 759.
Ralls, J. O. (1943). *J. biol. Chem.*, **151**, 529.
Skeggs, L. T. (1957). *Amer. J. clin. Path.*, **28**, 311.
Stubbs, J., and Pennybacker, J. (1960). *Lancet*, **1**, 1094.
Talso, P. J., Spafford, N., Blaw, M. (1953). *J. Lab. clin. Med.*, **41**, 281.
Van Slyke, D. D. (1947). *J. biol. Chem.*, **171**, 467.
—— and Hiller, A. (1947). Ibid., **167**, 107.

ENDOCRINE CONTROL OF SKELETAL DEVELOPMENT IN MAN*

BY

B. W. A. LEEMING, M.B., Ch.B., D.M.R.D. M.C.R.A.

Charge Radiologist, Hutt Hospital, New Zealand; Radiologist, Wellington Hospital, New Zealand

Exhaustive studies at the Institute of Experimental Biology, University of California, have shown that skeletal development in the rat entails two distinct processes each of which is under separate endocrine control. The first process, usually termed "bone growth," consists in the progressive increase in the dimensions of a bone. The dimension usually measured is length because of its simplicity. The second process is called "bone maturation," and this consists in the gradual acquisition of the adult shape of the particular bone. Maturation is assessed by radiological methods in clinical medicine. Apart from this experimental support there is also clinical evidence in favour of dissociated control of bone growth.

Evidence is produced here to show that in man at least three separate factors operate in skeletal development. Bone maturation as defined above is divisible into two separate elements, and these, together with the other element of bone growth, are each under different endocrine control.

Evidence from Experimental Animals.—The University of California workers (Becks *et al.*, 1946; Simpson *et al.*, 1950) demonstrated by their carefully controlled experiments on the rat that growth hormone chiefly increases the size of the skeleton and that thyroxine is mainly concerned with maturation, although it potentiates the action of growth hormone when used in combination. Alone, thyroxine has no effect on bone growth. Other hormones were found to have relatively little effect, although it was noted that oestrogens inhibit growth by antagonizing growth hormone, while androgens stimulate growth in the presence of the pituitary.

Clinical Evidence.—Pituitary disorders in the growing period of life are instructive. In gigantism the overacting hypophysis causes a marked acceleration of skeletal growth which does not stop at the usual time of puberty, since the epiphyses remain open. In infantilism the epiphyses again remain open but there is an almost complete absence of growth in length. It follows that the agent promoting growth does not also control epiphysial closure. The well-known acceleration

*Based on a paper read to the combined Sections of Orthopaedics and Radiology at the Annual Meeting of the British Medical Association, Auckland, New Zealand, 1961.

of growth terminated by premature maturation seen in hyperandrogenism and in the less common hyperoestronism does not provide evidence either for or against the hypothesis. Neither does hypothyroidism, since in this condition there is failure of growth and maturation together.

Evidence for the New Theory

Material.—The basis of this study consisted of a series of 25 radiographs of the hands and wrists of 23 cases of gonadal agenesis, together with sundry other radiographs, including 25 films of the pelvis. These films became available as a result of a comprehensive survey of 75 cases of Turner's syndrome and allied conditions presented by Bishop *et al.* (1960).

Of the 25 selected cases all except one regarded themselves as female and had been registered as female at birth. The exception had, of course, been registered as a male. Their ages ranged from 6 to 25 years. Certain tests of endocrine function had been also carried out, and a summary of the results is presented in modified form in Table I.

TABLE I

Case No.	B.M.R.†	17-KS.†	F.S.H.
13137	+1	−5	High*
90550	+4	+1	,,
76280	+1	−1	,,
31280		−2	,,
6885	−1	−4	,,
81705	−6	−7	,,
2934	+2	−5	,,
29990		−2	,,
88479		−5	,,
R.B.		−5	,,
11755		+3	,,
69208		−3	,,
57237	−6	−1	,,
22777	+21	−4	Normal
38794	+3	−5	,,
1288	−5	+4	,,
24804	−3	−5	High
23031		−4	,,
10554		−3	,,
4841	+1	−5	,,
8475	+1	−3	Normal
93421	+2	−4	High

B.M.R. = Basal metabolic rate.
17-KS. = 24-hour urinary output of 17-ketosteroids.
F.S.H. = Follicle-stimulating hormone.
* "High" represents any value more than 28 mouse units.
† The values given have been expressed in half standard deviations above or below the mean for the appropriate age group (2 half standard deviations = 1 standard deviation).

Technique

The hand and wrist films were examined in two ways. Firstly, the skeletal ages were computed, using the standards of Greulich and Pyle (1959), and the results were plotted against the chronological ages at the time of each of the *x*-ray films (Fig. 1). In two cases the patient had had films taken at different ages, but no distinction was made on this account and both estimations were included. Secondly, the length of the skeleton of each hand was measured to the nearest millimetre whenever possible. The length of the hand skeleton was taken to extend from the tip of the third terminal phalanx to the mid-point of the lower epiphysial plate of the radius. Any films in which such a measurement could not be made—as, for example, when the fingers were not straight or had not been entirely included on the film—were excluded from the series. The number of measurements recorded was 13. It was found necessary to use the radial epiphysial cartilage as an end-point, since the normal range of hand lengths against which these measurements were plotted extended from birth, at which time neither the carpal bones nor the wrist epiphyses are calcified. Furthermore, the radial epiphysis is an important element in estimations of bone age.

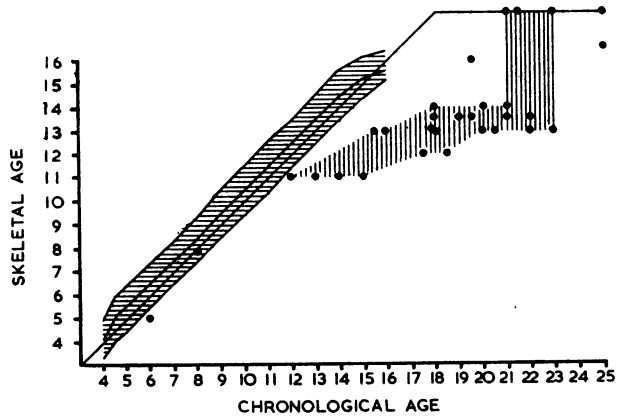

FIG. 1.—Normal range is expressed to the first standard deviation (horizontal lines). The straight diagonal line which extends horizontally represents the theoretical mean normal relationship between the two variables.

The reason why the length of the hand skeleton was used rather than total height for assessing growth rates was that it is known that the time of appearance and the rate of development of epiphyses in various parts of the skeleton differ in different individuals, and it is quite possible that growth in length may also proceed at differential rates in the same individual.

It became necessary to establish a normal range for hand lengths during growth. By the kind permission of Dr. J. M. Tanner I gained access to the hand *x*-ray films of about 2,000 normal individuals of both sexes, with a separate key to the age of each. After assessing hand length the results were plotted against age and were then statistically processed (Table II). The hand lengths of

TABLE II

Age	No. in Sample	Mean	Variation	Standard Deviation
		Females		
3 months ..	38	7·5368	0·1889	0·4346
6 ,, ..	40	8·1150	0·2182	0·4671
9 ,, ..	41	8·6085	0·1916	0·4377
12 ,, ..	26	9·1692	0·1598	0·3998
18 ,, ..	19	10·1316	0·2323	0·4820
2 years ..	37	10·5595	0·2591	0·5090
2½ ,, ..	12	11·19	0·2754	0·5248
3 ,, ..	19	11·66	0·3513	0·5927
3½ ,, ..	32	11·3813	0·2706	0·5202
4 ,, ..	17	12·1941	0·4481	0·6694
4½ ,, ..	24	12·3667	0·6075	0·7794
5 ,, ..	24	12·8042	0·3456	0·5879
6 ,, ..	48	13·2812	0·5122	0·7157
7 ,, ..	51	13·9353	0·4383	0·6620
8 ,, ..	75	14·3693	0·6319	0·7949
9 ,, ..	88	14·9809	0·5991	0·7740
10 ,, ..	70	15·5843	0·5906	0·7686
11 ,, ..	63	16·3875	0·8119	0·9011
12 ,, ..	59	16·9644	0·9251	0·9618
13 ,, ..	64	17·6641	1·0306	1·0152
14 ,, ..	39	17·9718	0·6126	0·7827
15 ,, ..	24	18·1625	1·3216	1·1496
		Males		
3 months ..	44	7·8159	0·2307	0·4803
6 ,, ..	53	8·4736	0·1804	0·4247
9 ,, ..	38	8·9342	0·3353	0·5791
12 ,, ..	24	9·3375	0·2311	0·4807
18 ,, ..	30	10·0467	0·3488	0·5906
2 years ..	36	10·6917	0·4351	0·6596
2½ ,, ..	12	11·28	0·2815	0·5306
3 ,, ..	20	11·61	0·3704	0·6086
3½ ,, ..	39	11·7846	0·3119	0·5585
4 ,, ..	22	12·2682	0·3832	0·6190
4½ ,, ..	18	12·5056	0·3323	0·5765
5 ,, ..	29	12·6828	0·4822	0·6944
6 ,, ..	39	13·4846	0·6498	0·8061
7 ,, ..	80	14·1912	0·3456	0·5879
8 ,, ..	90	14·8044	0·5294	0·7276
9 ,, ..	64	15·1656	0·7131	0·8445
10 ,, ..	65	15·7892	0·7882	0·8878
11 ,, ..	79	16·3544	0·4943	0·7031
12 ,, ..	70	17·0029	0·9075	0·9526
13 ,, ..	48	17·8812	1·0790	1·0387
14 ,, ..	46	18·4739	1·2824	1·1324
15 ,, ..	25	19·4880	1·3853	1·1770

the gonadal agenesis series were next plotted against the normal range so established. The resulting graph is produced in Fig. 2.

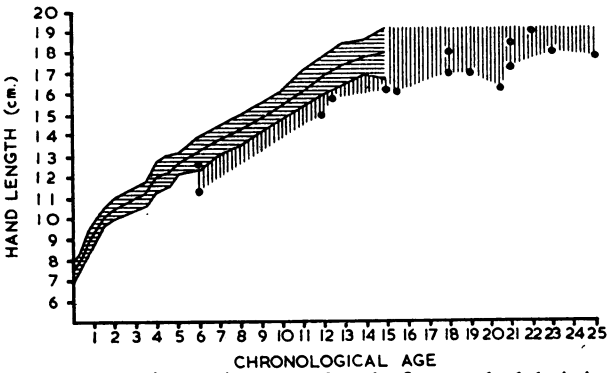

FIG. 2.—Normal range is expressed to the first standard deviation (horizontal lines).

In 25 cases views of the pelvic inlet were available, usually as part of a pyelogram. In studying these it was found that in 18 cases the pelvic inlet was android in shape, with a triangular outline and flattened walls (Moloy, 1951). It was thought that this was a significantly high number, but it was felt necessary to check on the validity of this observation since the pelvic views might have been distorted owing to technical radiographic factors. Therefore 100 consecutive pyelograms were studied at Guy's Hospital by an independent observer (Dr. Ian Kerr). He marked each pelvic inlet as either male or female without prior knowledge of the actual sex of each subject and he subsequently compared his assessment against the real sex. He correctly predicted the sex from pelvic radiographs in 92% of the cases. Fig. 3 shows why this accuracy is possible. Because of the inclination of the pelvic inlet and the position of the *x*-ray source the *x* rays passing through the inlet are almost at right angles to it and so there is little distortion.

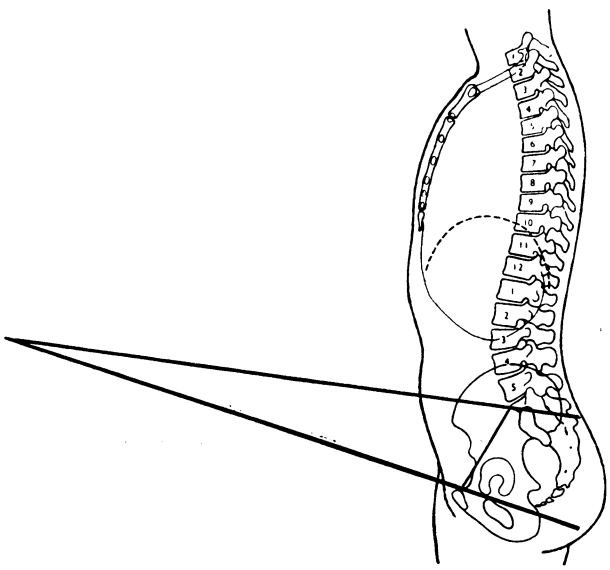

FIG. 3.—Divergent lines represent a beam of *x* rays passing through the pelvic inlet. For full explanation see text.

Results and Interpretations

Skeletal Age

Fig. 1 shows the normal range to the first standard deviation of bone age from the seventh to the seventeenth year. This part of the graph was derived from figures published by Greulich and Pyle (1959) and forms the area crossed by the horizontal lines. The range for the agenesis cases is represented by the area covered by the vertical lines.

It is unfortunate that the under-12 years group is represented only twice, but this is a retrospective study of a randomly selected group. Nevertheless, these two examples fit with various previously published studies in which there is no material deviation from the normal until the twelfth year (Grumbach *et al.*, 1955; Van der Werff ten Bosch, quoted in commentary by Bishop *et al.*, 1960). After the twelfth year the curve flattens out and the bone age becomes relatively static at a value of 13 to 14 years until after the twentieth year, when the digital epiphyses finally unite.

It was really this remarkable difference between the behaviour of the normal individual and that of the cases of gonadal dysgenesis with respect to skeletal development that first led to the work embodied in this paper.

Hand Length

The results in this section are quite different. Fig. 2 shows the normal range to the first standard deviation (horizontal lines) and the curve for the agenesis series (vertical lines). Both curves are similar in shape, but in the case of the latter the curve is lower in position. This fits well with most published reports on total height. These children grow at a normal rate, but at any age they are somewhat shorter than their normal fellows (Albright *et al.*, 1942; Wilkins *et al.*, 1944; Grumbach *et al.*, 1955; Hoffenberg and Jackson, 1957; Bishop *et al.*, 1960).

The significance of these findings can be interpreted as follows. In the normal child growth and maturation proceed in harmony. In those diseases causing growth retardation both factors usually fail together. The result is that evidence in favour of dissociated control is hardly ever obvious. Gonadal dysgenesis is the exception to this in that only one element fails, and so the required evidence is revealed.

Referring again to the two graphs, three components are discernible. The second graph (Fig. 2) shows the factor of bone growth as already defined. The first curve (Fig. 1) can be divided into two distinct components. Since it is derived from measurements entirely different from those in the other graph, there must be three components in all. These components are thought to be: (*a*) bone growth—or increase in skeletal dimensions; (*b*) bone maturation—or development of final shape; and (*c*) epiphysial closure—the third process.

Discussion

When sought for, evidence of dissociation can be found in atlases of skeletal development such as that of Greulich and Pyle. The first phase of post-natal bone development ends during the twelfth year, and after this time the development of the shape of carpal and finger bones progresses no further. Instead, the epiphyses show a progressive fusion with the shaft. Now the assessment of bone age has been devised on an empirical basis, and ageing is determined by whatever changes occur, irrespective of their cause. The fact is, however, that it would not be a difficult matter to describe the course of development in terms of maturation and epiphysial fusion and to provide norms of skeletal age on such a basis. If this were done the assessment of skeletal age would not become less accurate, and in addi-

tion there would be the advantage of having more information about the possible cause of the abnormality in the individual case. It is possible that with new methods of analysis of skeletal development, such as that of Acheson (1954), these diverse elements will be more adequately recognized. Owing to the small number of the endocrine assays (Table I) no attempt will be made to place responsibility for control of two of these factors, and the role of growth hormone and thyroxine can be expected to be deduced from future studies. The figures in Table I may be of interest to others and are for this reason included.

In the case of control of epiphysial closure, certain evidence is available from a study of the shape of the pelvic inlet. It was stated earlier that in 18 cases out of 25 the inlet was triangular in outline, while in the remainder it was either rounded in outline or the shape was indeterminate. This high proportion of so-called android pelves cannot be due to an increased androgen level, since the 17-ketosteroid level is reduced. It must be concluded, therefore, that the triangular pelvic inlet is not due to any abnormal endocrine stimulus at all. It can be explained thus. The pelvic brim in both sexes is triangular before puberty, and this change persists in the normal male after puberty. In the normal female, however, the pubertal rise in oestrogens causes a change in the pelvic shape which will fit her for future childbirth. In this the pelvis can be compared to the mammary gland, in that both are indifferent to circulating androgens but respond to oestrogens.

In ovarian agenesis this triangular pelvis is almost certainly due to the low oestrogen level. It is suggested that this low level is more likely to be the cause of the marked delay in the closure of the epiphyses than the slight reduction in the androgen level.

Furthermore, such a conclusion is in harmony with the high F.S.H. values, which presumably also indicate low oestrogen levels in females.

Summary

There is good evidence, derived from animal experiments and from clinical experience, that skeletal development is ultimately controlled by the endocrine system and that this control is multiple.

A study of the radiographs of the hands of 25 cases of ovarian agenesis, together with the films of the pelvic inlets of these cases, led to the conclusion that there are three components in bone growth—namely, (1) assumption of final dimensions, (2) assumption of final shape, and (3) epiphysial closure—and that these three elements are under separate endocrine control. These conclusions were arrived at by estimating both the bone age and the length of the hand skeleton in each case and plotting the results against the normal range for each measurement.

As a result of a study of the pelvic films and of certain endocrine assays which were available, it was also concluded that, in the female, epiphysial closure is controlled by oestrogens.

A new measurement is presented—namely, skeletal hand length, which is referred to simply as hand length throughout this paper. Tables are included which give statistical details for both sexes covering a large number of age-groups.

Acknowledgments are due to Dr. P. M. F. Bishop, department of endocrinology, Guy's Hospital, London, for permission to make use of material supplied by him; to Dr. John Tanner, Institute of Child Health, London, for providing the material used to compile the table of normal hand lengths; to Dr. Ian Kerr for investigating the shape of the pelvic brim as seen in routine pyelograms; and to Dr. David Reid for helpful criticism in drafting the paper. I am also indebted to Mr. T. Roberts of the Applied Mathematics Division, D.S.I.R. Wellington, New Zealand, for statistically processing the data on hand length.

REFERENCES

Acheson, R. M. (1954). *J. Anat. (Lond.)*, **88**, 498.
Albright, F., Smith, P. H., and Fraser, R. (1942). *Amer. J. med. Sci.*, **204**, 625.
Becks, H., Collins, D. A., Simpson, M. E., and Evans, H. M. (1946). *Anat. Rec.*, **94**, 631.
Bishop, P. M. F., Lessof, M. H., and Polani, P. E. (1960). Memoirs of the Society for Endocrinology, No. 7, Sex Differentiation and Development, p. 162.
Greulich, W. W., and Pyle, S. I. (1959). *Radiographic Atlas of Skeletal Development of the Hand and Wrist*, 2nd ed. Stanford Univ. Press, California.
Grumbach, M. M., Van Wyk, J. J., and Wilkins, L. (1955). *J. clin. Endocr.*, **15**, 1161.
Hoffenberg, R., and Jackson, W. P. N. (1957). *Brit. med. J.*, **2**, 1457.
Moloy, H. C. (1951). *Clinical and Roentgenologic Evaluation of the Pelvis in Obstetrics*. Saunders, Philadelphia and London.
Simpson, M. E., Asling, C. W., and Evans, H. M. (1950). *Yale J. biol. Med.*, **23**, 1.
Wilkins, L., and Fleischmann, W. (1944). *J. clin. Endocr.*, **4**, 357.

PROBLEMS AND MANAGEMENT OF HERMAPHRODITISM

BY

J. I. FORBES, M.B., M.R.C.P.Ed.
Physician

G. E. CLAYDEN, M.B., B.Ch.
Plastic Surgeon

AND

B. HAMMAR, M.B., F.R.C.S.Ed.
Surgeon

Harari Hospital, Salisbury, Southern Rhodesia

The sense of being a complete male or female is happily taken for granted by the vast majority of human beings. To the unfortunate few who are aware that they properly belong to neither sex the problem of intersex is the cause of a great deal of distress and embarrassment, and until recently it has been the cause of considerable confusion in the mind of the medical adviser.

Two main factors appear to enter into the mechanism of the differentiation of sex: firstly, there is the genetic factor which determines the sex of the embryo and of its gonads; and, secondly, there is the influence of the sex hormones produced by these gonads. Harrison (quoted by Gillies and Millard, 1957) says:

> "One of the remarkable facts about the development of the reproductive organs, both internal (the gonads) and external (the genitalia), is that young embryos possess organs which at first give no evidence as to whether they will become those of a male or of a female. Thus there can be considered to be a 'neuter' or 'indifferent' stage of the embryo's early development, during which the external genitalia of embryos, destined to be either male or female, look alike. It is as if the forces which will eventually bring about the 'maleness' or 'femaleness' of the embryo