removing vasoconstrictor nervous tone. A chemical cause for the vascular narrowing thus demonstrated seemed out of the question, in view of the much lower arterial pressures recorded in the legs, where Lewis had found a normal blood-flow; and the vascular narrowing above the aortic constriction was attributed to a local change. Steele (1941) has since shown, by direct recording, that the diastolic arterial pressure is often nearly as high below the aortic constriction as above it, thus removing the argument against a humoral mechanism. A further similarity with essential hypertension is to be found in Friedman, Selzer, and Rosenblum's (1941) finding of a reduced effective renal blood-flow and normal glomerular filtration rate, inferred from the diodrast and inulin clearances.

NEPHRITIS

Chronic.—Vasoconstriction of non-nervous origin has been demonstrated in the skin of the hand in chronic nephritis as in essential hypertension (Pickering, 1935-6b). No information is available concerning muscle or cerebral flow; reduced blood-flow through the kidneys has been inferred (Chesley *et al.*, 1940), but there is no information as to what vessels are involved and how. Allegations that the hypertension behaves differently in chronic nephritis and essential hypertension have on repetition been unconfirmed (Pickering and Kissin, 1935-6; Pickering, 1935-6b).

Acute.—While all the varieties of chronic hypertension which I investigated behaved similarly in showing evidence of vasoconstriction in the hand after removal of sympathetic vasoconstrictor tone, acute nephritis behaved differently (Pickering, 1935-6c). During the phase of hypertension the blood-flow (as reflected by calorimetry) was in the upper normal range or above it, and in 4 of 6 cases was significantly greater than when the arterial pressure had returned to normal. The increase in maximum heat elimination from the hand produced by a given rise in arterial pressure—all other known factors, including the tone of the hand vessels, remaining constant—had been determined in a case of femoral arteriovenous fistula in which compression of the artery proximal to the fistula raised diastolic arterial pressure by 15 mm. Hg. In the four cases of acute nephritis the increase in heat elimination per unit rise of diastolic pressure was quite similar (Fig. 2); there was thus

FIG. 2.—Shows the relationship between diastolic pressure and the heat elimination from the hand, from which vasoconstrictor nervous tone had been removed, in 4 cases of acute nephritis (solid circles, solid triangles, solid squares, open triangles) and in a case of arteriovenous fistula (open circles).

no evidence of non-nervous vasoconstriction of the hand vessels during the phase of hypertension. It was concluded, therefore, that the mechanism of raised arterial pressure in acute nephritis differed from that in chronic hypertension,* and that while the latter was probably due to a chemical agent the former probably was not. Similar results in 5 cases were obtained by Arnott and Matthew (1939). While no observations by

* It will be seen that in acute nephritis the "control" is provided by the same patient after recovery, and thus the factor of individual variation is excluded. I have had only two opportunities of measuring the heat elimination from the hand after removing the vasoconstrictor tone in different stages of chronic hypertension, and these support the conclusions already described. Thus in a case of progressive chronic nephritis arterial pressure was 138/80 and heat elimination 525 cals. per min. on Feb. 6, 1936; on Sept. 12, 1936, the corresponding figures were 146/96 and 480. In a case of essential hypertension passing from the benign to the malignant phase arterial pressure was 195/124 and heat elimination 281 cals. per min. on Nov. 1, 1935; on June 9, 1936, the corresponding figures were 234/144 and 290.

the most modern methods have yet been published on the renal circulation in acute nephritis, Goldring and Smith's preliminary results, published in 1937, "suggest that renal hyperaemia may exist during a phase of acute glomerular nephritis." Unfortunately they did not state whether hypertension was present in this phase.

Published records give us a very incomplete picture of the circulation in nephritic hypertension, but, so far as they go, they suggest that in chronic nephritis the circulation behaves in much the same way as it does in essential hypertension, and is probably likewise humoral in origin. In acute nephritis the hypertension seems to arise from a different cause. If a chemical agent is involved it is peculiar in that it constricts neither the vessels of the hand nor apparently those of the kidney. A nervous mechanism is possible, though none has been demonstrated.

PREGNANCY TOXAEMIA

In a brief summary of work done before the war Kellar and Sutherland (1941) state that the heat elimination from the hand after elimination of vasomotor nervous tone was normal in 35 cases of toxæmia, but give no details. The renal circulation has been investigated by Corcoran and Page (1941), Chesley *et al.* (1940), and by Wellen, Welsh, and Taylor (1942), who are in substantial agreement. The glomerular filtration rate and the tubular excretory mass tend to be normal, while the effective renal blood-flow is increased. It is suggested that these changes are the result of efferent glomerular dilatation with possibly thickening of the glomerular basement membrane.

In pregnancy toxæmia, therefore, the circulation apparently presents some features similar, and some dissimilar, to acute nephritis. We must await further evidence to decide.

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(Lecture II, with a list of references, will appear next week.)

THE DIFFERENTIAL DIAGNOSIS OF SPINAL ARTHRITIS IN YOUNG SUBJECTS*

BY

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The symptoms of arthritis of the spine in young subjects may present a diagnostic problem of considerable difficulty and at the same time of the greatest importance in view of the rapid progress and grave developments in the more serious forms.

The Typical Form

The form most commonly seen in young people is that which is associated with the names of Marie and Strümpell, although its characteristic features had been described many years earlier by Hilton Fagge (1877) and others. The descriptive name of "ankylosing spondylitis" is now generally used; but Gilbert Scott (1942) advocated the name "adolescent spondylitis," as it usually begins at that period of life. Its rapid development and relentless progress to complete rigidity of spine, hips, and thorax, and later other joints, have been described elsewhere (Buckley, 1931). The earliest symptoms are vague pains in the back and limbs; sometimes at the outset they may be chiefly complained of in the limbs, but low back pain usually appears soon and is associated with flattening of the normal lumbar curve. At this stage the condition may be confused with adolescent kyphosis, but in the latter the gradually increasing curvature of the dorsal spine is usually associated with a compensatory increase of the normal lumbar curvature. As the disease progresses, the gait and facies are characteristic and striking.

The sequence of events is synovitis and inflammatory hyperaemia of the apophyseal joints of the lumbar spine and usually of the sacro-iliac joints, followed very soon by implication of the costo-vertebral joints and extension to the dorsal spine. Osteoporosis of the bones forming these joints appears at the

* Based on a communication to the East Midlands Society of Physicians at Leicester, April 3, 1943.

same time, and may possibly be the primary change; this is probably due to hyperaemia of the bone substance and a raised pH, leading to absorption of calcium, which in turn tends to be re-deposited in the adjacent tissues where the pH is normal—mainly in the ligaments, joint capsules, and periosteum. The rarefying process rapidly spreads to the vertebral bodies, while the cartilage of the affected joints is gradually destroyed, the destructive process spreading from the periphery of the joints towards the centre. The intervertebral disks do not become thinned, but, on the contrary, sometimes expand from the turgor of the disks tending to compress the softened bone of the vertebral bodies. The apophysial joints and also the sacro-iliacs become ankylosed, and the calcification involves the ligamenta flava, which are in close relation to the joints, and extends to the other vertebral ligaments. The disease spreads from joint to joint, always in a centrifugal manner, missing none in the acute form of adolescence—first to the hips and costo-vertebral articulations, the sterno-clavicular and mandibular often before the knees and shoulders—but it generally ceases to extend before the smaller joints are reached. If the hands or feet are affected before or simultaneously with the spine the diagnosis is not ankylosing spondylitis, but infective arthritis, possibly of gonorrhoeal origin, affecting the spine.

Other Forms

While this is the typical form, spondylitis of a rather different character is often met with at a slightly later age—between 30 and 40 is usual. It is possible that this may have begun in earlier life in a mild form and undergone spontaneous arrest, to be roused again by strain, exposure, or other causes lowering the general resistance. I have met with several cases in the Forces. The condition is characterized by the fact that the changes in the apophysial joints are less pronounced and often absent in the mid-lumbar region; the sacro-iliac joints are only partially ankylosed or may escape, but the lower dorsal spine and the dorso-lumbar region show definite changes, partial or complete bridging of adjacent vertebrae by ossification of the ligaments being commonly present. Osteoporosis is less marked than in the adolescent variety and may not occur. The sex incidence seems to be different, and though males are still the more often affected, there is less disparity between the sexes. The disease is slower in development and often does not progress beyond the dorsal spine. Gold, which in my experience and that of many others is ineffective in the adolescent form, sometimes appears to do good in these cases. It is probable that these are the cases which have been described by Knaggs (1926) and others under the name "spondylosis ossificans ligamentosa" and which are characterized by systematic ossification of the ligaments but no changes in the apophysial joints or the disks. The dorsal spine is chiefly affected; the cervical and lumbar regions are less often attacked and mobility is not seriously interfered with, any loss of mobility being generally due to involvement of the apophysial joints. There are indications that the condition begins as a fibrositis of the spinal ligaments and not in the joints. Stockman (1926) states that "ossifying spondylitis" is due to the formation of new bone in chronically inflamed fibrous tissue, but the cases he records appear to include examples of both types described above and also cases of definitely infective origin. Fluorine poisoning has been shown to produce similar changes, generally appearing at an earlier age—about puberty: mottling of the dental enamel is a distinguishing feature.

Oppenheimer (1938), who has made an extensive study of the various forms of spinal arthritis, stresses the fact that ossification of ligaments is a very common and totally uncharacteristic response to a great variety of intervertebral lesions—inflammatory, degenerative, destructive, or traumatic. It is unfortunately the case, however, that the least sign of bony change in the spinal column is commonly labelled spondylitis, and the patient draws the conclusion that he is the victim of a chronic progressive crippling disease.

A disorder which may easily be confused with the early stages of adolescent spondylitis has been described by Rogers and Cleaves (1935) as "the adolescent sacro-iliac joint syndrome." They reported a group of cases which showed symptoms of low back stiffness, pain and tenderness over the sacro-iliac joints, often with sciatic pain and loss of straight-leg raising, sometimes unilateral, in others affecting both sides. They

regard the condition as an epiphysitis, and point out that it may be confused with osteomyelitis, tuberculosis, or arthritis. These epiphyses appear about the fifteenth year and join up about the age of 20. It is probable that this condition is the same as that to which the name "sacro-iliitis" has been given and which has been erroneously regarded as the invariable precursor of ankylosing spondylitis. With rest and appropriate measures it tends to recover completely, and it seems probable that this result has led to the idea that certain methods of treatment applied in such cases have been responsible for the supposed arrest and cure of ankylosing spondylitis in its early stage. There may be some blurring of the line of the sacro-iliac joints in such a condition, and patchy osteoporosis, but not of the degree seen in spondylitis; and the apophysial joints are unaffected. It is analogous with the vertebral epiphysitis, described by Steindler (1929) and others, which occurs in adolescence and affects the epiphyses of the epiphysial ring that appear about the age of 14 and join up from 22 to 25 and is often the precursor of adolescent kyphosis.

Time of Onset

The onset of ankylosing spondylitis at an age when bone growth is active is probably responsible for some of its pathological characters, and it is remarkable that it frequently attacks robust and athletic youths rather than the weakly. It often happens that a history of an accident when playing is forthcoming, but in view of the frequency of such accidents it is doubtful how far they are of aetiological significance.

Another important feature is that the disease appears at the time when the sex glands are undergoing rapid development: the preponderance in the male sex suggests that the prostatic secretion may have some significance in view of the fact that it is rich in phosphatase, which has considerable influence on calcium metabolism—a possibility calling for further investigation.

It is believed by many authorities that ankylosing spondylitis is simply rheumatoid arthritis attacking the spine, but from this view I must differ. The predominance in the male sex, the much greater tendency to ankylosis than in rheumatoid arthritis, the strictly centrifugal spread, and the lack of any effective relief from gold are all strongly against the rheumatoid theory; and other grounds might be stated. Whether the form appearing in adult life is related to rheumatoid arthritis or originates as a form of fibrositis is a question less easily settled. It has been found that the tuberculin-sensitiveness is about 30% higher in the subjects of ankylosing spondylitis than in a series of rheumatic control cases without evidence of active tuberculosis (Robinson, 1940).

X-ray Appearances

It should be an invariable practice in all cases of pain in the lower back and limbs in young subjects to have an x-ray examination without delay. A history of vague and fleeting pains in adolescents should not be lightly regarded, for such symptoms may go on for months before anything serious is suspected. In some cases the limb pains predominate and the back pains are later in appearing. While antero-posterior and lateral views of the lumbar spine and pelvis may reveal some changes, especially in the region of the sacro-iliac joints, the most important view to be taken is a three-quarter lateral, at an angle of 45 degrees, which in most people is the only one that will show the lower lumbar apophysial joints. A loss of density in the articular processes, together with a blurring of the outlines of the joints, is among the earliest signs of spondylitis, and if associated with such symptoms as mentioned above and an accelerated sedimentation rate the diagnosis is reasonably certain.

Treatment

Rest is imperative in the early stages, preferably in bed on a hard mattress; if any tendency to kyphosis has begun to appear a plaster shell, on the lines described by Pemberton and Osgood (1934), may be necessary. As the more acute symptoms subside a plaster jacket may be applied, reaching from the upper part of the spine to below the iliac crests, as advised by Swaim (1939); this will need to be worn for months and be followed by a spinal brace. Care must be taken to provide an ample diet of milk and other sources of essential vitamins and calcium, and cod-liver oil is of much value. Heliotherapy

and ultra-violet irradiation will help to improve calcium metabolism, while infra-red rays often give much relief from pain. Breathing exercises are of the greatest importance in preventing, so far as is possible, the tendency of the costo-vertebral joints to ankylose; they also have a beneficial effect on the general condition. Deep x-ray therapy has been credited with good results in some cases. I have not found drugs of any service except for relief of pain, for which aspirin, or in severe cases tab. codein. co., will be useful. Gold, as already stated, is quite ineffective in the adolescent form, and the same may be said for vaccines.

BIBLIOGRAPHY

- Buckley, C. W. (1931). *British Medical Journal*, 1, 1108.
 — (1938). *Arthritis, Fibrositis, and Gout*, Lewis, London.
 Comroe, B. I. (1940). *Arthritis and Allied Conditions*, Kimpton, London.
 Fagge, C. H. (1877). *Trans. path. Soc.*, 28, 201.
 Kemp, F. H., Murray, M. M., and Wilson, D. G. (1942). *Lancet*, 2, 93.
 Knaggs, R. L. (1926). *The Inflammatory and Toxic Diseases of Bone*, Bristol.
 Oppenheimer, A. (1938). *J. Bone Jt. Surg.*, 20, 285.
 Pemberton and Osgood (1934). *The Medical and Orthopaedic Management of Chronic Arthritis*, Kimpton, London.
 Robinson, G. (1940). *Ann. rheum. Dis.*, 2, 41.
 Rogers, M. H., and Cleaves, E. N. (1935). *J. Bone Jt. Surg.*, 17, 759.
 Scott, S. Gilbert (1942). *Adolescent Spondylitis*, London.
 Steindler, A. (1929). *Diseases and Deformities of the Spine and Thorax*, London.
 Stockman, R. (1926). *Edinb. med. J.*, 33, 577.
 Swaim, L. T., *J. Bone Jt. Surg.*, 21, 983.

A STUDY OF THE ACCURACY OF SERUM PROTEIN ESTIMATIONS AND OF DIURNAL VARIATIONS IN THEIR LEVEL*

BY

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The object of the present investigation was: (i) to estimate the degree of accuracy obtainable in our hands with two methods of serum protein estimation in common use; (ii) to determine whether there is any diurnal variation in the level of serum proteins in the normal individual under ordinary conditions of activity and food intake.

Methods

Serum was used in preference to plasma for the following reasons: (a) haemolysis is less likely to occur in serum than in plasma; (b) there is a theoretical possibility that the presence of oxalate may, by withdrawing water from the cells, dilute the plasma, while the presence of heparin, which contains nitrogen, must make any estimation of protein inaccurate; (c) in another investigation in progress, necessitating the estimation of proteins, it is more convenient to use serum than plasma. It was therefore preferable to have a base-line in terms of serum than of plasma proteins. The methods used were:

(i) Proteins were precipitated with zinc sulphate and caustic soda and estimated by the micro-Kjeldahl method as described by Pregl (1937) and Niederl and Niederl (1938). In the samples taken at two-hourly intervals the result recorded represents the mean of two determinations. (ii) The method described by King and his co-workers (King, Haslewood, and Delory, 1937; King, Haslewood, Delory, and Beall, 1942), in which Kjeldahl digestion is followed by Nesslerization and colorimetric estimation. The colorimetric readings were made with a Hellige colorimeter in a dark-room. Owing to war conditions a Klett violet filter, as advised in the original paper, was unobtainable; an Ilford blue filter was therefore used. In the samples taken at two-hourly intervals the result recorded represents the mean of two determinations.

Results

Accuracy of the Method.—To test the accuracy of the method it would have been satisfactory to estimate the protein content of 100 samples of the same serum on the same day. Owing to the time and apparatus required for the micro-Kjeldahl method this was not practicable. Ten estimations were therefore made on each of 10 samples of the same serum by both methods. This procedure was carried out for 10 different sera, giving 100 estimations in all (Table I). These figures were treated in two different ways to determine what difference in any two results should be attributed to technical error. First working on the basis that any two of these values observed for a given serum might have occurred in actual

practice, the distribution of differences between the 10 values was determined. Each 10 results thus gave 45 differences (or 90 if read both ways). There were in all, therefore, 450 differences. Each 45 based on a separate experiment. The number of differences of various sizes was very similar for each of the 10 sera, though the

TABLE I.—Estimations from Ten Samples of the Same Serum, using King's Method and the Micro-Kjeldahl Method. Estimated with 10 Different Sera

Serum	King's Method			Micro-Kjeldahl Method		
	Mean Reading	Mean Difference	Max. Difference	Mean Reading	Mean Difference	Max. Difference
1	7.48	0.14	0.3	7.40	0.056	0.14
2	7.23	0.18	0.4	6.79	0.054	0.16
3	6.91	0.16	0.4	6.69	0.086	0.22
4	6.27	0.17	0.4	6.26	0.069	0.17
5	6.45	0.24	0.5	6.38	0.067	0.18
6	7.10	0.16	0.4	6.80	0.049	0.14
7	6.06	0.16	0.4	6.03	0.070	0.18
8	6.97	0.14	0.4	6.95	0.053	0.14
9	6.66	0.09	0.2	6.67	0.072	0.22
10	6.76	0.10	0.3	6.75	0.079	0.21

average value of the latter varied from 6.06 to 7.48 with King's method and from 6.03 to 7.4 with the micro-Kjeldahl method. Within this range it seems, therefore, that errors of observation will be of the same magnitude. Adding them together for the 10 sera gives the figures shown in Tables II and III. With King's method (Table II) it seems that differences as large as 0.3 and even 0.4 will

TABLE II: KING'S METHOD.—Grouped Differences in Value of Serum Proteins, using 10 Samples of the Same Serum and 10 Different Sera, compared with Similar Differences observed on 5 Samples withdrawn at Two-hourly Intervals from Each of 20 Normal Subjects

Differences in g.	Differences between Estimations on the Same Serum		Diurnal Variations	
	No. of Diffs. Observed	% Frequency	No. of Diffs. Observed	% Frequency
0.0	113	25	47	25
0.1	121	27	78	41
0.2	123	27	32	17
0.3	50	11	25	13
0.4	37	8	8	4
0.5	6	1	1	0.5
0.6	—	—	1	0.5

TABLE III: MICRO-KJELDAHL METHOD.—Grouped Differences in Value of Serum Proteins, using 10 Samples of the Same Serum and 10 Different Sera, compared with Similar Differences observed on 5 Samples withdrawn at Two-hourly Intervals from Each of 20 Normal Subjects

Differences in g.	Differences between Estimations on the Same Serum		Diurnal Variations	
	No. of Diffs. Observed	% Frequency	No. of Diffs. Observed	% Frequency
0.00	175	39	58	30
0.05	171	38	59	31
0.10	77	17	40	21
0.15	20	4	22	12
0.20	7	2	6	3
0.25-0.35	—	—	7	4

occur quite frequently with the same serum, for 11% of them were 0.3 and 8% were 0.4. A difference as large as 0.5 is rare, for there was only 1 in 100. With the micro-Kjeldahl method, on the other hand (Table III), it appears unlikely that differences over 0.2 will be obtained in testing the same serum (only 2 in 100), and fairly unlikely that any over 0.15 (only 6 in 100) will occur. As a satisfactory rule, according to this method of analysis, changes of under 0.15 should be ignored with the micro-Kjeldahl method as too likely to be due to errors inherent in the technique, changes of 0.15 to 0.2 as probably significant, and those over 0.2 as almost certainly significant.

The same figures were treated in a different manner with approximately the same results. An average standard deviation from the total 100 values was obtained by calculating the sum of the squared deviations from the observed means in each set of 10 tests on the same serum, adding all these squares together, and dividing by 90 (the number of degrees of freedom left). This standard deviation from the 100 values for King's method was 0.1416 and for the micro-Kjeldahl method 0.0574. The test of significance between any two observed values was then measured by the standard deviation multiplied by $2\sqrt{2}$. Differences as large as this value would be expected to occur by chance only once in 20 trials. With King's method, therefore, the difference between two values has to be at least 0.4 and with the micro-Kjeldahl technique at least 0.16 to be significant.

Either method of treating the figures shows that the micro-Kjeldahl technique is the more accurate.

* A report to the Medical Research Council from the N.W. London Blood Supply Depot.