Effect of Peritoneal Dialysis.—In two patients carbenicillin was administered during peritoneal dialysis and the serum levels and half-life were compared with those obtained in these two patients when they were not being peritoneally dialysed. Peritoneal clearances of carbenicillin were obtained in four consecutive exchange periods in each patient. Table IV shows the results of serum levels and half-life obtained during peritoneal dialysis in these two patients and Table V shows the carbenicillin concentrations obtained in the peritoneal dialysis fluid and the corresponding peritoneal clearances of carbenicillin. In Case 5 there was a reduction in half-life from 6.7 hours without dialysis to 4.2 hours during peritoneal dialysis. In Case 4 the half-life was reduced from 23 hours without dialysis to 7.4 hours during peritoneal dialysis. The mean peritoneal clearance of carbenicillin in these two patients was 6.8 ml./min.

Side-effects.—None were observed during this study.

TABLE IV.—Effect of Peritoneal Dialysis on Carbenicillin Levels

Case No.	Dose	Serum Carbenicillin Levels (μ g./ml.) During Peritoneal Dialysis					Half-life (Hours)
		Zero	1 Hr.	4 Hr.	8 Hr.	24 Hours	
4	1 g.	< 3.0	115	97	63	13.7	7.4
5	1 g.	< 3.0	82	42	36	—	4.2

TABLE V.—Carbenicillin Concentrations in Peritoneal Dialysis Fluid with Corresponding Peritoneal Clearances of Carbenicillin for Four Consecutive Exchanges in Two Patients

Case No.		Exchange Period			
		1	2	3	4
4	Carbenicillin conc. (μ g./ml.) ..	29	34	47.5	28
	Peritoneal carbenicillin clearance (ml./min.) ..	6	8	12	6
5	Carbenicillin conc. (μ g./ml.) ..	20	18	15	12
	Peritoneal carbenicillin clearance (ml./min.) ..	5	4	6	8

Discussion

The mean half-life of carbenicillin in those patients with endogenous creatinine clearances of less than 5 ml./min. was found to be 13.1 hours. There was, however, a wide range of from 6.7 to 23.5 hours. The mean serum level at one hour after the administration of 2 g. of carbenicillin intravenously was 207 μ g./ml. To maintain serum carbenicillin levels of

around 100 μ g./ml., therefore, it would seem necessary to give 2 g. of carbenicillin intravenously every 8 to 12 hours. As the drug appears to be relatively free of side-effects, 2 g. of carbenicillin intravenously every eight hours might be employed.

During haemodialysis with the two-layer Kiil dialyser and using cuprophane membrane PT150, the half-life of carbenicillin in three patients was reduced. During haemodialysis, therefore, carbenicillin should be given at more frequent intervals and 2 g. intravenously every four hours is suggested.

The peritoneal clearance of carbenicillin was poor, with a mean clearance of 6.8 ml./min., though there was a reduction in half-life of from 6.7 to 4.2 hours in one patient and from 23 to 7.4 hours in another during peritoneal dialysis. During peritoneal dialysis 2 g. of carbenicillin intravenously every six hours is suggested.

Summary

The serum half-life of carbenicillin in patients with severe renal failure was determined. The mean serum half-life in eight patients was 12.5 hours.

Haemodialysis with the two-layer Kiil dialyser and using cuprophane membrane PT150 produced a reduction in half-life in three patients.

Peritoneal clearance of carbenicillin was poor, with a mean clearance of 6.8 ml./min. in two patients.

To maintain serum carbenicillin levels of around 100 μ g./ml. in patients with severe renal failure—that is, endogenous creatinine clearance of <5 ml./min.—a dosage schedule of 2 g. intravenously every eight hours is suggested. This regimen requires some modification during haemodialysis or peritoneal dialysis.

We would like to thank Sister J. M. Storey and the staff of the intermittent dialysis unit, Fulham Hospital, who collected some of the samples. Carbenicillin was supplied by Dr. E. T. Knudsen, of Beecham Research Laboratories, Brentford, Middlesex. Mr. R. Sutherland, of Beecham Research Laboratories, Brockham Park, Betchworth, Surrey, carried out the carbenicillin biological assays.

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Preliminary Communications

Adrenergic Myenteric Nerves in Hirschsprung's Disease

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The clinical picture of Hirschsprung's disease is due to disordered function of a segment of colon and rectum. The histological abnormalities of this segment are an absence of myenteric ganglion cells and the presence of large abnormal nerve trunks (Whitehouse and Kernohan, 1948). These nerve trunks give a strong acetylcholinesterase staining reaction (Kamijo *et al.*, 1953). The myenteric ganglia are usually accepted as part of the parasympathetic system, and their absence may account for the disordered peristalsis in Hirschsprung's disease. It has also been suggested that a deficient

adrenergic innervation might be responsible for the contracted state of the affected bowel (Kamijo *et al.*, 1953; Wright and Shepherd, 1965). In the present study we have tested this possibility using the catecholamine fluorescence technique of Falck and Hillarp (Falck, 1962).

METHOD

The abnormal bowel was resected because of Hirschsprung's disease in five children aged from 4 months to 3½ years. Normal control specimens were obtained from two children, aged 11 and 15 months, whose lower bowel (including the rectum from one) had been resected for other reasons. Small full-thickness pieces of gut from different levels of each resected specimen were rapidly frozen, freeze-dried, and treated with formaldehyde gas as described by Falck and Owman (1965).

The specimens were then embedded in paraffin, sectioned at 8 μ , and examined by fluorescence microscopy with an Osram HBO 200 lamp and BG 12 filters. Adjacent pieces of tissues were treated similarly, but without the formaldehyde gas, to act as controls for non-specific fluorescence. In all instances alternate serial sections were stained with haematoxylin and eosin or cresyl violet to aid structural identification.

RESULTS

In the test tissues pale green fluorescent adrenergic nerves were readily recognizable. The control tissues showed no auto-fluorescent granules in ganglion cells or muscle cells. No non-specific connective-tissue fluorescence occurred which could be misinterpreted as adrenergic nerves in the muscle layers or would interfere with the appearances of these nerves in the formaldehyde-treated material. This contrasts with the complication caused by non-specific fluorescence in adult human tissues which has been observed in the gut (Baumgarten, 1967) and salivary glands (Garrett, 1967).

NORMAL COLON

The adrenergic innervation of the normal infant colon was similar to that in experimental animals (Norberg, 1964; Jacobowitz,

1965) and normal human adults (Baumgarten, 1967). Adrenergic nerves were sparse in the muscle layers, but characteristic strongly fluorescent basket-like arrangements of nerves appeared to surround myenteric ganglion cells (see Fig. 1). No fluorescent ganglion cells were seen.

HIRSCHSPRUNG'S DISEASE

Proximal Region.—In three specimens the unconstricted proximal part had a normal histological appearance, but in two of these there were fewer adrenergic nerves surrounding the ganglion cells than in the controls.

Aganglionic Region.—In all of the diseased specimens there were many more adrenergic nerves in the muscle layers of the distal constricted segment of the gut than in the more proximal regions or in any part of the control specimens. In two cases the muscular adrenergic innervation in the constricted segment was extensive and in another it was vast (see Fig. 2); in this one case there were more adrenergic nerves in the circular muscle than in the longitudinal muscle, but in the other four the reverse appeared to be true. Neither ganglion cells nor the basket-like arrangement of fluorescent nerves were seen. In the zone between the longitudinal and circular muscle layers there were many adrenergic nerves, from which the nerves in the muscle itself often appeared to emanate. The large nerve bundles, which are a feature of this disease, were not fluorescent except for an occasional adrenergic nerve running through a bundle. It was not uncommon, however, to find medium-sized fluorescent nerve bundles in the intermuscular zone.

Transitional Region.—In three of the cases tissue from the transitional zone showed mixtures of large nerve bundles and small scattered groups of non-fluorescent ganglion cells partly surrounded by the beginnings of a basket-like fluorescence. Some of the medium-sized nerves in the intermuscular zone were fluorescent, but the large nerve bundles were non-fluorescent except for an occasional fibre running through them.

DISCUSSION

The results with normal colon confirm that the sympathetic nerves occur mainly in association with intramural non-adrenergic ganglia, and that few are present in the muscle layers. The aganglionic colon in Hirschsprung's disease, however, contained many adrenergic nerves, especially in the muscle layers. This increase was most marked in the two children who presented with intestinal obstruction in the neonatal period. The constriction of the aganglionic zone therefore cannot be due to the absence of sympathetic tissue as has sometimes been postulated. The presence of adrenergic nerves and the absence of myenteric ganglia suggest that a different interpretation of physiological and pharmacological observations in this disease is necessary. For example, rectal distension in the normal patient inhibits the activity of the internal anal sphincter and causes it to relax, whereas in Hirschsprung's disease this response is absent (Lawson and Nixon, 1967). A similar defect was confirmed in the present series by one of us (E. R. H.). It seems likely that in normal colon the myenteric ganglia are involved in this inhibitory response. Pharmacological studies in vitro with the use of nicotine to stimulate nervous tissue have shown that whereas normal colon is relaxed the aganglionic and transitional segments are either contracted or unaffected (Wright and Shepherd, 1965; Bennett and Howard, 1967). It would thus seem that in the normal colon the relaxant effect of nicotine involves the ganglia. This at first seems surprising, because the ganglia have usually been thought of as the part of the parasympathetic system which induces gut contraction.

How then does the normal colon relax, and how does nicotine exert its normally relaxant effect? It would appear that a

FIG. 1.—Normal rectum showing complex arrangements of fluorescing adrenergic nerves in three myenteric ganglia lying between the sparsely innervated muscle layers (circular, above; longitudinal, below). Some submucosa is included in the top left-hand corner. ($\times 56$.)

FIG. 2.—Aganglionic rectum from a case of Hirschsprung's disease showing numerous fluorescing adrenergic nerves in the muscle layers (circular, above; longitudinal, below) and in the intermuscular zone, but the large nerve bundles in this zone are non-fluorescent. ($\times 56$.)

release of noradrenaline is involved, since adrenergic blocking drugs prevent relaxation (Bucknell and Whitney, 1965). One possibility is that noradrenaline exerts an inhibitory effect on the ganglia (Norberg, 1964; Jacobowitz, 1965). This idea is consistent with the presence of α -adrenergic receptor sites within neurones in the gut (Kosterlitz and Watt, 1965). Another possibility involves non-adrenergic inhibition, which has been found in animal and human intestine (Burnstock *et al.*, 1964; Bucknell, 1966). Some of the ganglia might take part in this inhibition and their absence would prevent the colon from relaxing.

SUMMARY

The histological distribution of adrenergic myenteric nerves has been studied by the catecholamine fluorescence technique in large bowel resected from young children, five because of Hirschsprung's disease and two for other reasons. Normal large bowel showed a dense arrangement of adrenergic nerves around myenteric ganglion cells. None of the ganglion cells contained catecholamines. Few adrenergic nerves were seen in the muscle layers. In Hirschsprung's disease the myenteric ganglion cell complex was absent from the distal segment of bowel, but increased numbers of adrenergic nerves were present in the muscle layers. The large nerve bundles which are characteristically seen in the intermuscular zone in this disease were non-fluorescent, but some medium-sized bundles were fluorescent. Transitional tissue, which shows features intermediate between this and the normal bowel, was seen in three cases. These findings indicate that inability of the affected segment to relax is not due to an absence of adrenergic nerves.

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Medical Memoranda

Goldenhar's Syndrome

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Goldenhar (1952) reviewed a symptom-complex of ocular, aural, and vertebral defects. This is usually referred to as the ocular-auriculo-vertebral syndrome of Goldenhar. Though this syndrome is rare, a number of cases have now been reported. Certain characteristic manifestations have been described and incomplete cases are also found, such as those without vertebral anomalies (Sugar, 1966) and those without the auricular anomalies (Weyers and Thier, 1958); these probably all belong to the same symptom-complex. The case reported is another fairly typical example of this syndrome.

CASE REPORT

This male infant was born at term after a normal pregnancy and labour. He weighed 7 lb. 15 oz. (3,600 g.) at birth. His parents were healthy (father aged 25 and mother aged 21). There was no history of any maternal illness during pregnancy. When first examined the face was asymmetrical; there were multiple skin tags on the right side (Fig. 1) and two preauricular tags on the left side (Fig. 2). The lower jaw was maldeveloped and the right ramus of

the mandible could not be palpated. The right external meatus was occluded by skin. There was a coloboma of the left upper eyelid at the junction of the middle and inner thirds. Both pupils were small and the corneae appeared to be large. Radiological examination confirmed the absence of the right ramus of the mandible and the lateral third of the zygomatic arch (Fig. 3). The vertebral column was normal. Examination under anaesthesia at 3 months of age confirmed that both corneae were large (11 cm.), but the

FIG. 1.—Skin tags on right side of face and coloboma in left upper eyelid.

FIG. 2.—Left preauricular skin tags.