At times a lack of correspondence has been observed between the two methods. On the one hand, significant radioactivity has been found with negative benzidine tests (as in days 3 and 4 of Case 2); and on the other, weakly positive benzidine reactions have been observed with no significant radioactivity in the faecal samples. These discrepancies have always been when the amount of blood was small as indicated by one test or the other.

Discussion

Quantitative chemical methods for determining the amount of blood present in faeces are laborious and inexact (Andrews and Oliver-González, 1942). For this reason very little information is as yet available about the daily blood loss in many common diseases.

The radio-isotope method is simpler, and its general validity was carefully worked out in experiments with dogs by Owen *et al.* (1954a). They labelled dogs' own red cells with ^{51}Cr , placed known quantities of this labelled blood in the dogs' stomachs, then measured the faecal radioactivity and obtained very good correspondence. They also found that in dogs with ^{51}Cr -labelled red cells the amount of ^{51}Cr passing into the bowel in the absence of bleeding was less than 0.1% of the original dose daily, equivalent to less than 1 ml. of blood daily. A larger amount (1% a day) was excreted in the urine. They concluded that the method can reasonably be applied to man (Owen *et al.*, 1954b; Cooper *et al.*, 1955).

The studies reported here confirm their view that the ^{51}Cr method provides a simple reliable way of quantitatively estimating faecal blood loss. Our experience with patients in whom chronic or subacute gastro-intestinal bleeding was known or suspected indicates that the method may be of considerable value not only in research but also in diagnosis. It may give evidence not only of the quantity of blood lost daily, but also sometimes an indication of whether the site of bleeding is high or low in the gastro-intestinal tract.

The accuracy of the ^{51}Cr method described is clearly least when the blood is in high dilution, because the radioactivity of the stool sample becomes nearer background activity. The present findings show that the method is not capable of measuring accurately very small amounts of blood—that is, of the order of 3 ml. or less—in an average stool. It is thus probably less sensitive in detecting bleeding than is the Gregersen benzidine test. This is a limitation of the method which must be accepted, for, although it could be overcome by using a larger dose of ^{51}Cr , a dose greater than 300 μC is probably not justified (Mollison and Veall, 1955).

The present study also confirms the conclusion of Needham and Simpson (1952) on the value of the modified Gregersen benzidine test. They found that a minimum of 3 to 5 ml. of blood administered by stomach-tube was necessary to produce a positive (+) result. In the present study + results were obtained with stools of average bulk (50–200 g.) when they contained 3–14 ml. of blood as estimated by the ^{51}Cr method. The Gregersen benzidine test is, of course, not intended to be quantitative, and, as pointed out above, the gradations in intensity noted have relatively little significance unless the bulk of the stool is taken into account. For example, on different occasions in this study a blood loss of 10 ml. has been associated with benzidine test results of $\pm$, +, and ++. Further, as Needham and Simpson pointed out, occasional false positives are obtained, and the test is most useful when it is repeated on a series of samples.

Summary

A method using tracer doses of ^{51}Cr for the quantitative estimation of gastro-intestinal blood loss is described. Detailed results from three patients are given to illustrate the value of the method in expressing clinical observations in quantitative terms, and sometimes in indicating the possible site of bleeding. A comparison is made between the findings with this method and results of a commonly used qualitative benzidine occult blood test.

On the whole there is good correlation between the tests. The chromium method has least value when blood loss is 3 ml. or less in the stool samples analysed.

I am grateful to Dr. G. Wetherley-Mein for his advice and encouragement, and to Dr. J. L. Pinniger for allowing me facilities in the Louis Jenner Laboratory and for reading the manuscript. I thank the physicians of St. Thomas's Hospital for permission to investigate patients under their care.

ADDENDUM.—Since submitting this paper for publication the technique has been much simplified by the introduction of a modified turret counter of the type described by Veall (Veall and Vetter, 1952). This was constructed by the hospital Physics Department. Each stool is collected into a large wax carton which can be placed directly in the counter. The activity of the whole sample can thus be determined without the elaborate manipulations described in the text.

REFERENCES

- Andrews, J. S., and Oliver-González, J. (1942). *J. Lab. clin. Med.*, **27**, 1212.
Cooper, M., and Owen, C. A., jun. (1956). *Ibid.*, **47**, 65.
— Stroebe, C. F., Stickney, J. M., and Owen, C. A., jun. (1955). *Fed. Proc.*, **14**, 31.
Gerritsen, Th., Heinz, H. J., and Stafford, G. H. (1954). *Science*, **119**, 412.
Gray, S. J., and Sterling, K. (1950). *J. clin. Invest.*, **29**, 1604.
Mollison P. L., and Veall, N. (1955). *Brit. J. Haemat.*, **1**, 62.
Needham, C. D., and Simpson, R. G. (1952). *Quart. J. Med.*, **21**, 123.
Owen, C. A., jun., Bollman, J. L., and Grindlay, J. H. (1954a). *J. Lab. clin. Med.*, **44**, 238.
— Cooper, M., Grindlay, J. H., and Bollmann, J. L. (1954b). *Proc. Surg. Forum. Clin. Congr. Amer. Coll. Surg.*, **5**, 663.
Veall, N., and Vetter, H. (1952). *Brit. J. Radiol.*, **25**, 85.
Wetherley-Mein, G., Hutt, M. S. R., Langmead, W. A., and Hill, M. J. (1956). *Brit. med. J.*, **1**, 1445.

LEUKAEMIA, AMYLOIDOSIS, AND RENAL VEIN THROMBOSIS IN IRRADIATED ANKYLOSING SPONDYLITIS

BY

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Interest has recently been stimulated in two syndromes—namely, leukaemia following irradiation for ankylosing spondylitis (Court Brown and Abbott, 1955; Abbott and Lea, 1956) and renal vein thrombosis following amyloidosis (Harrison *et al.*, 1956). A case, in our experience unique, is reported showing both syndromes.

Clinical History

A store manager aged 32 was discharged from the Army in 1945 with a diagnosis of ankylosing spondylitis. From 1945 to 1948 he was treated by deep x rays to his spine. The total dose received was about 1,200 r. He was subsequently encased in a spinal jacket, which he had worn for the nine months previous to his first presentation at our hospital in June, 1948.

He then complained of pain in the back of both thighs and in the back on sitting down. His spine was completely rigid and movement of the hips was limited. The Wassermann and Kahn reactions were negative, the E.S.R. was 40 mm., and the x-ray examination was typical of spondylitis. He removed to another area and from 1948 to 1950 he was treated at another hospital with further deep x rays to the spine to a total dosage of between 1,000 and 1,500 r.

In 1953 he had renal symptoms for the first time. At one hospital he complained of left and then right renal colic,

and his renal function was found to be poor. This was confirmed at another hospital, where he was shown to have a high blood urea and anaemia with a normal blood pressure. Chronic nephritis possibly due to irradiation was diagnosed, and he was treated with multiple transfusions.

In February, 1954, he was readmitted to our hospital with renal symptoms. He was "clinically anaemic," the blood pressure was 140/90, the fundi were normal apart from pallor, and the only oedema he showed was a slight swelling of the left ankle. The remainder of the clinical examination revealed nothing abnormal. The blood urea was high (156 mg./100 ml.) and gradually rose throughout the year to his death in October (390 mg.). On several occasions between February and September, 1954, the serum proteins were between 4 and 5%, with the albumin about 2.5%. During the same period several urine examinations showed a severe albuminuria, few white and red cells, but no casts. A blood count in February, 1954, showed: red cells, 1,900,000 per c.mm.; Hb, 41% (6.07 g./100 ml.); white cells, 5,700 per c.mm. (polymorphs 68%, eosinophils 4%, lymphocytes 25%, monocytes 2%, basophils 1%). Anisocytosis and poikilocytosis were severe, and polychromatophilia and basophil stippling of the red cells were present. He was given multiple transfusions and discharged.

He was readmitted to our hospital on September 27, complaining of vomiting, sometimes "black," for six days, and severe backache. On examination he was obviously very ill, and was very pale, with ulceration of his throat. He was tender over the spine and other bones. The glands were not enlarged and the liver, spleen, and kidneys were not palpable. His blood urea was 390 mg. per 100 ml.; he had a severe albuminuria and the serum proteins were reduced. A blood count showed: red cells, 2,700,000; Hb, 47% (6.9 g./100 ml.); white cells, 13,600 (polymorphs 7%, eosinophils 1%, lymphocytes 18%, monocytes 1%, "blasts" 73%). Severe anisocytosis and poikilocytosis were present; the red cells were small and pale, with a few larger polychromatic cells. Platelets were scanty. The peroxidase stain of the "blasts" was negative. Acute leukaemia was diagnosed, and it was thought to be lymphatic in type. A marrow examination confirmed this, 87% of the cells resembling lymphocytes or lymphoblasts.

The patient had various further haemorrhagic episodes, and he died on October 8, 1954.

Post-mortem Examination

A thin young man with dark-brown pigmentation of the skin except in the bathing-trunk area. *Bones*:—Ankylosing spondylitis. Rich grey-red marrow in femur, sternum, and vertebrae. *Heart and vessels*:—Normal apart from the renal veins, which are described below (see "kidneys"), and occasional scattered atheromatous plaques in the aorta. *Respiratory tract*:—Mucous haemorrhages in nose and nasopharynx with ulceration. Oedema of both lung bases. *Alimentary tract*:—Multiple erosions in lower oesophagus and in stomach, where they were widely scattered. Much altered and fresh blood in stomach and throughout the bowels. *Liver*:—Swollen, pale, smooth, and shiny. *Spleen and lymph nodes*:—Spleen normal size, firm, even, reddish brown. Slight generalized enlargement of lymph nodes. *Thyroid, parathyroids, adrenals, pancreas*:—Normal. *Skull, meninges, brain, cord, and middle ears*:—Normal.

Left Kidney (11 by 6 by 4 cm. Weight 126 g.).—Aberrant renal artery at lower pole. Cortex 10 mm. deep, pale, with small haemorrhagic areas. Medulla also pale. Pelvis normal. Capsule thickened and did not strip readily. Surface finely granular, pale, with spider areas upon it. Renal arteries appeared to have normal walls and lumen. Marked narrowing, thickening, and distortion of the renal vein near the kidney after commencing normally. Calcified areas in the narrowed portion of the renal vein, its branches, and in the medulla.

Right Kidney (9 by 4.5 by 2.5 cm. Weight 70 g.).—Cortex 5 mm. deep, pale, with darker radial streaks. Medulla

appeared normal apart from calcified areas mentioned later. Calcified areas in linear form apparently following vessels in the pelvic area, while other similar calcified areas were present in the medulla. Capsule thickened, not stripping readily, leaving a finely granular pale surface with spider areas. Renal arteries apparently normal. Renal vein commenced normally, but was completely obliterated near the kidney, appearing as a thickened firm cord with calcified areas.

Post-mortem Histology.—*Renal arteries and veins extra-renal*:—Arteries normal, but calcified thrombus in renal veins. *Kidneys*:—Severe amyloid change. Extensive small round-cell infiltration of leukaemic type. Veins in pelvis and medullae were obliterated by old thrombi, which were frequently calcified. The arteries were clear. *Liver, spleen, and adrenals*:—Severe amyloid change. *Marrow and lymph nodes*:—Lymphatic leukaemic change. *Heart and stomach*:—Normal.

Post-mortem Radiography.—The kidneys were radiographed after removal at necropsy, and both showed an unusual calcification—the left more than the right—linear in type and most pronounced at and near the pelvis. This confirmed the calcification noted at necropsy and showed that it was more extensive than at first appeared.

Discussion

This complicated case, showing ankylosing spondylitis, for which irradiation was given, amyloidosis, "chronic nephritis," renal vein thrombosis, and leukaemia, was difficult to explain at the time it occurred. Since then papers having a bearing on certain aspects of this case have made apparent the probable course of events.

Amyloidosis is known to occur in occasional cases of ankylosing spondylitis (W. St. C. Symmers, 1957, personal communication), and there is evidence that renal vein thrombosis may occur in amyloid disease (O'Beirn, 1953; Harrison *et al.*, 1956). The patient's clinical "chronic nephritis" would appear to have been due to amyloidosis and renal vein thrombosis. The thrombosis had to a large extent organized, with resultant sclerosis, calcification, and loss of lumen of the renal veins. What part, if any, the irradiation played in the production of the thrombosis is difficult to assess. The dose of x rays was not excessive, and renal complications of irradiation have not often been reported. The most important series of cases of renal damage following radiotherapy is that of Luxton (1953), who described 27 cases irradiated for seminoma. Of these, 13 developed symptoms of acute nephritis, 12 of chronic nephritis, and 2 of malignant hypertension. Similar cases have been reported by other workers, and Smith and Wynn Williams (1955) described in detail, with a full necropsy report, a further case of irradiation nephritis.

It must be emphasized that all the cases we have seen reported received a comparatively heavy dosage of x rays for malignant conditions varying from about 3,000 to 6,000 r in four to five weeks. This is in marked contrast to our own case, in which the dose has been calculated not to have exceeded 500 r at the medial surface of the kidney and 100 r at the renal parenchyma over a period of three years. In our experience such a dosage has not been known to produce renal damage. Furthermore, vascular damage caused by irradiation is arterial and is an endarteritis obliterans while the veins remain clear. In our case thrombosis, sclerosis, and calcification occurred in the veins, the arteries apparently being spared. X rays may have been partly responsible for the renal-vein lesions, but we think this unlikely. It is more likely that they followed the amyloidosis.

The accumulation of evidence that leukaemia is apt to occur in cases of ankylosing spondylitis given irradiation continues, and Abbatt and Lea (1956) have theorized on the possibilities at play. Although the x-ray dosage in our case was within reasonable limits, the possibility that the leukaemia followed the irradiation is undeniable. The leukaemia has usually been described as an acute myeloblastic type in the reports. We considered our case to be

an acute lymphoblastic leukaemia, being, of course, fully aware of the difficulty of classifying very primitive and atypical cells.

The series of related events in our case might be expressed as :

Summary

A case of ankylosing spondylitis complicated by amyloid disease and renal vein thrombosis is described. Treated by irradiation, the patient subsequently developed acute leukaemia, probably lymphatic, and succumbed. The interrelation of the various conditions is discussed.

We wish to thank Dr. S. A. Probert for permission to publish the case and Professor W. St. C. Symmers for his helpful criticism.

REFERENCES

- Abbott, J. D., and Lea, A. J. (1956). *Lancet*, 2, 1317.
 Court Brown, W. M., and Abbott, J. D. (1955). *Ibid.*, 1, 1283.
 Harrison, C. V., Milne, M. D., and Steiner, R. E. (1956). *Quart. J. Med.*, 25, 285.
 Luxton, R. W. (1953). *Ibid.*, 22, 215.
 O'Beirn, S. (1953). *Irish J. med. Sci.*, p. 181.
 Smith, W. G., and Williams A. Wynn (1955). *Lancet*, 2, 175.

ENTEROPATHOGENIC ESCHERICHIA COLI SEROTYPES: INFECTION OF NEWBORN THROUGH MOTHER*

BY

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The epidemiology of gastro-enteritis caused by the enteropathogenic *Escherichia coli* serotypes has hitherto been studied, with exceptions, in paediatric clinics and nurseries (Taylor *et al.*, 1949; Rogers and Koegler, 1951; Rogers, 1951; Taylor, 1951; Taylor and Charter, 1952; Ocklitz and Schmidt, 1954; Adam, 1956; Rogers and Cracknell, 1956). Outside these establishments it has been difficult to follow the chain of infection (Braun, 1956).

In the children's hospital or ward the primary infection usually originates in the sick babies, but occasionally adults in the nursing staff are carriers of the germ (Ocklitz and Schmidt, 1955), although not ill. The spread of the infection in hospitals may also be caused through contamination of food of babies during preparation (Ocklitz and Schmidt, 1954).

Contact between infants outside hospital is possible too; in Germany the babies are often assembled in the waiting-rooms of the child welfare institution, and they may get infected in the consultation-room. There may also be cross-infection from infant to infant in the family

*Paper, in extract, read by one of us (H. W. O.) at the eighth International Congress of Paediatrics in Copenhagen, Denmark, July, 1956.

itself. But all these factors fail to explain the relatively high percentage of healthy carriers among the infants in the general population.

During earlier investigations we found pathogenic *E. coli* in 5.8% of the infants who had not had contacts with infected babies (Ocklitz and Schmidt, 1954). These results correspond with those of other authors (Taylor, 1953; Braun, 1956). In explaining such results, it is again necessary to consider the part played by the contamination of food. This leads us to the person who cares and cooks for the baby every day—namely, the mother—and her epidemiological role in *E. coli* diarrhoea.

We have tried to examine the factors which link the mother and her baby, especially the newborn baby, in this infection. In our examination we identified pathogenic strains of the well-known *E. coli* O groups 111, 55, 26, 86, and 44, and the type O25:L11:H6, which we isolated in a ward of our hospital during a severe epidemic of coli-enteritis (Ocklitz and Schmidt, 1952). We can only briefly deal with serological methods, referring to other more detailed literature (Kauffmann, 1954; Kauffmann and Ørskov, 1956).

Material and Method

Cultures were made from the faeces and lochia of women, lochial specimens being collected on throat swabs. From newborn infants rectal swabs were taken, using a sterile Drigalski spatula, care being taken to obtain traces of faeces. Media were inoculated at once at the patient's bedside. To avoid a confluent growth we inoculated several culture dishes, using Endo, Drigalski, and Leifson media (Schmidt, 1956). At the same time the antibiotic resistance was tested.

After 18 hours' incubation, and after the exclusion of the *Salmonella* and *Shigella* groups of bacteria, the *E. coli* colonies were tested with diagnostic sera by way of slide agglutination. We examined at least 10 single colonies or a sample of the bacterial growth, using two polyvalent sera of the following *E. coli* types:

Serum I: O111:B4:H-; O55:B5:H-; O26:B6:H-.

Serum II: O25:L11:H6; O86:B7:H34; O44:L74:H18.

These sera were so composed as to avoid overlapping reactions of their antigens. In this way a preliminary diagnosis was made which was confirmed in further tests. Having taken pure cultures, we started with the tube agglutination. In this agglutination we tested separately the O and B antigens. At the same time the strain was examined in a U-tube for its motility in semi-stiff medium (Kauffmann, 1954). If the strain was motile we identified the H antigen by means of the immobilization test, using H sera (Kauffmann and Ørskov, 1956).

Results

Mothers in the maternity wards of the gynaecological clinic of the University of Rostock were examined. In 22 (8.8%) of the 250 specimens, pathogenic *E. coli* serotypes were isolated from the stools or lochia before or after the delivery (Ocklitz and Schmidt, 1955) (see Table I). The fact

TABLE I.—Results of Examination of Women in the Gynaecological Clinic

Specimens	Cases	Negative Proof (No Growth on Endo Medium)	Serotypes of <i>E. coli</i>				Total
			O111: B4	O55: B5	O26: B6	O25: L11	
Last stool before delivery ..	100	—			1	6	7
First stool after delivery ..	100	—	3			11	14
Lochial smear ..	50	15	1				1