APPLICATION OF THE RADIO-IODINE ROSE BENGAL TEST IN LIVER DISEASE IN INFANCY AND CHILDHOOD*

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Since the introduction of the radio-iodinated rose bengal liver-function test by Taplin *et al.*[1] numerous studies, carried out on adults, have indicated the usefulness and limitations of this method in the diagnosis of liver disease. Since radio-iodinated (^{131}I) rose bengal is selectively taken up from the blood by the polygonal cells of the liver, following its intravenous administration, and excreted in the intestines, the test may yield useful information concerning, *inter alia*, the following: (*i*) Size, shape and position of the liver; (*ii*) the detection of intrahepatic lesions (e.g. tumours, abscesses, cirrhosis, hepatitis); (*iii*) the distinction between obstructive and non-obstructive jaundice; and (*iv*) the rate of clearance of the isotope from the blood and the uptake by the liver which give some indication of liver function.[2]

The value of the radio-iodinated rose bengal method in distinguishing between obstructive and non-obstructive jaundice is well established.[2-6] In recent years the value of this method in differentiating between biliary atresia and 'neonatal hepatitis' in young infants has been assessed in several studies.[7-9] The difficulty in distinguishing between these 2 entities was discussed recently.[9] Infants with either of these conditions present with obstructive jaundice, and it is generally agreed that differentiation on clinical and biochemical grounds is in most instances quite impossible.[9]

Several pathologists are of the opinion that the histological features of biliary atresia and 'neonatal hepatitis' are so distinctive that liver biopsy almost invariably permits a correct diagnosis.[10,11] Others believe that biopsy will often, but by no means invariably, permit a definite diagnosis.[10,12,13] It has recently been shown in a large series that the diagnostic accuracy of biopsies on specimens obtained by needle puncture or by an open technique was about the same, with only approximately 60% of infants with biliary atresia or 'neonatal hepatitis' being correctly diagnosed by either method.[12] An operative diagnosis of extrahepatic biliary atresia is often inaccurate.[14,15] In a recent study even operative cholangiography suggested a diagnosis ultimately shown to be wrong in 20% of cases.[12] For these reasons an assessment of the diagnostic value of the radio-iodine rose bengal method in liver disease in infants and children, particularly in cases of obstructive jaundice, may be of some interest.

PATIENTS AND METHODS

A total of 67 liver scans were carried out on most of the patients suffering from various forms of liver disease who were admitted to our paediatric wards during the past 2½ years (Tables I - IV). The method, which was described in more detail in a previous publication,[9] can be summarized as follows: All the patients received Lugol's iodine several hours before the test was carried out, to ensure chemical block of the thyroid. A dose of 15 μc of ^{131}I-labelled rose bengal was then given intravenously. The first scan was made over the heart, liver and abdomen by means of a Picker magna-scanner V, about 30 minutes after the injection, and repeated after approximately 3, 6 and 24 hours. When deemed necessary, e.g. in cases showing delayed excretion of the isotope by the liver, or absence of activity over the intestinal area (suggesting complete biliary obstruction), the scan was usually repeated daily up to 5 days. Radioactivity should show in the small intestine after 20 minutes, and after 60 minutes the total radioactivity in the small intestine should surpass that of the liver.[9] With normal function, the liver should contain no labelled dye after 24 hours.[9]

With few exceptions blood counts, conventional liver-function tests and serologic tests for syphilis were carried out on all the patients. When deemed necessary the following investigations were done: tests to exclude specific disease entities, e.g. infectious mononucleosis and toxoplasmosis, blood cultures, reducing substances in the urine, and cytological examination of the epithelial cells in urinary sediment for the presence of cytomegalic inclusion bodies. In addition, the 32 livers were examined histologically after obtaining biopsy specimens by needle puncture, an open procedure or necropsy.

RESULTS

The different forms of liver disease encountered are given in Tables I - IV. Although the radio-iodine rose bengal test was not performed on every patient, the complete list is given as the incidence of the various entities encountered may be of some interest.

TABLE I. CAUSES OF LIVER DISEASE IN BANTU INFANTS (0 - 2 YEARS)

Diagnosis	*Number of cases*
Infectious hepatitis	12
'Neonatal hepatitis'	8
Syphilitic hepatitis	8
Biliary atresia	5 + ? 1
Sepsis	3
Biliary cirrhosis	2
Veno-occlusive disease	3
Venous congestion	1
Miliary tuberculosis	1
Rupture of liver	1
Cause not established	7
Total	52

TABLE II. CAUSES OF LIVER DISEASE IN WHITE INFANTS (0 - 2 YEARS)

Diagnosis	*Number of cases*
'Neonatal hepatitis'	5
Cirrhosis (following 'neonatal hepatitis')	1
Infectious hepatitis	1
Fatty liver (cause unknown)	1
'Inspissated bile syndrome' (following ABO-incompatibility)	1
Total	9

*Date received: 8 May 1968.

TABLE III. CAUSES OF LIVER DISEASE IN BANTU CHILDREN (2 - 12 YEARS)

Diagnosis	*Number of cases*
Infectious hepatitis	20
Chronic venous congestion	2
Liver abscess (? amoebic)	1
Primary hepatoma	1
Metastases (Wilms's tumour)	1
Cause not established	4
Total	29

TABLE IV. CAUSES OF LIVER DISEASE IN WHITE CHILDREN (2 - 12 YEARS)

Diagnosis	*Number of cases*
Infectious hepatitis	32
Infectious mononucleosis	1
? Veno-occlusive disease	1
Cause unknown	1
Total	35

Liver scanning was found to be of particular value as an aid in the diagnosis of parenchymatous lesions of the liver, and in the differentiation of extrahepatic biliary atresia from intrinsic liver disease causing obstructive jaundice.

The test was performed on several patients with infectious hepatitis and cirrhosis. The typical patchy appearance (Fig. 1) was noted on occasion, but the method was not of particular diagnostic value in these cases as there was usually no difficulty in diagnosing these conditions clinically or by other means.

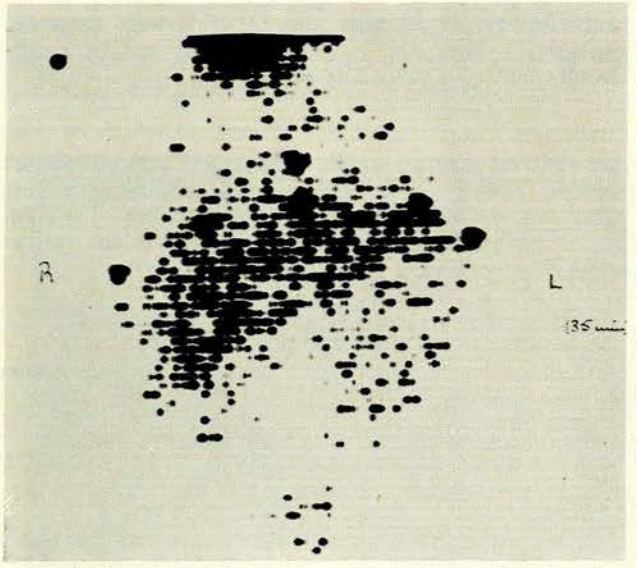

Fig. 1. Liver scan of an infant with cirrhosis, showing the typical patchy appearance. Considerable activity is still present over the heart area, indicating retarded uptake of ^{131}I by the liver from the blood.

The usefulness and limitations of the method are illustrated by the following case reports.

Parenchymatous Lesions

Case 1. J.J., a 9-year-old Bantu boy, became progressively thinner and weaker during the 4 months before admission. He also complained of abdominal pain and loss of appetite. On examination he was emaciated and there were signs of malnutrition (hyperpigmentation and scaling of the skin). The liver was tender and hard and extended 10 cm. below the costal margin.

A Mantoux test was negative, a chest X-ray was normal and the LE phenomenon was negative. He had a mild hypochromic anaemia. Serial liver-function tests were indicative of liver damage (increased serum γ-globulin, strongly positive thymol flocculation and turbidity tests, and increased transaminase and lactic acid dehydrogenase activity). The radio-iodine rose bengal test showed multiple defects in the liver, particularly in the right lobe (Fig. 2). The most likely cause was considered to be metastases from a neuroblastoma or nephroblastoma, but biopsy of a specimen obtained by needle puncture revealed primary hepatoma (Fig. 3).

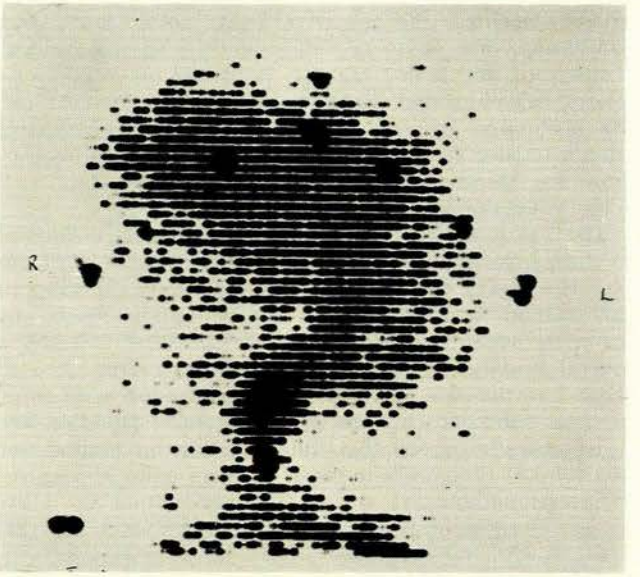

Fig. 2. Liver scan of 9-year-old Bantu patient (case 1) with primary hepatoma of the liver showing multiple defects, particularly in the right lobe. The gallbladder is visualized and appears to be displaced medially. Considerable activity is present over the intestinal area.

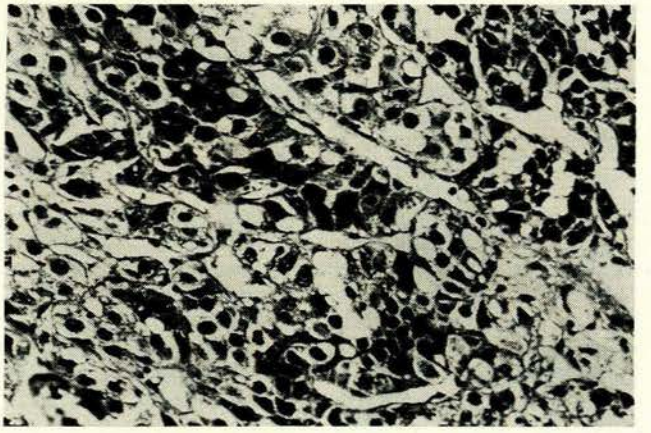

Fig. 3. Needle biopsy (case 1) showing histological features of hepatocellular carcinoma.

A course of 5-fluorouracil injections was given and considerable improvement occurred. He was discharged, but failed to return for follow-up examination.

Case 2. B.K., a 5-year-old Bantu girl, complained of abdominal distension and constipation which began about 3 weeks before admission. On examination she appeared anaemic and had hyperpigmentation and scaling of the skin. The liver reached the level of the umbilicus and was tender and smooth.

Radiological examination of the chest was normal and the Mantoux test negative. Intravenous pyelography showed the right kidney to be displaced inferiorly by the enlarged liver, but there was no distortion of the calyces. Apart from a slightly elevated total serum bilirubin level (1·2 mg./100 ml.) and a prolonged prothrombin time (60% of normal) liver-function tests were normal. A liver scan indicated a large defect in the region of the right lobe (Fig. 4). A lateral scan showed the cold area to be situated posteriorly (Fig. 5).

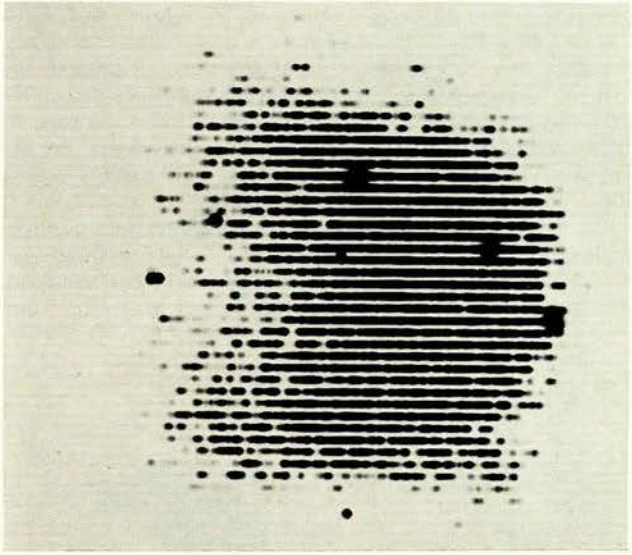

Fig. 4. Liver scan (case 2) showing large defect in the region of the right lobe, probably due to an amoebic abscess.

Because of the appearance of the liver scan and a fluctuating temperature, a diagnosis of an abscess was made and the patient was transferred to a surgical ward. The abscess was aspirated, but unfortunately the fluid did not reach the laboratory. Anti-amoebic treatment was instituted and the patient improved rapidly. On discharge, about 6 weeks after admission, the size of the liver had decreased to about 3 cm. below the costal margin.

Obstructive Lesions

The vast majority (50 of the 61 patients listed in Tables I and II) of young infants with liver disease presented with obstructive jaundice. The radio-iodine rose bengal method was found to be a valuable aid in distinguishing between extrahepatic biliary atresia and other forms of intrinsic liver disease causing obstructive jaundice. In most of the infants with 'neonatal hepatitis', syphilitic

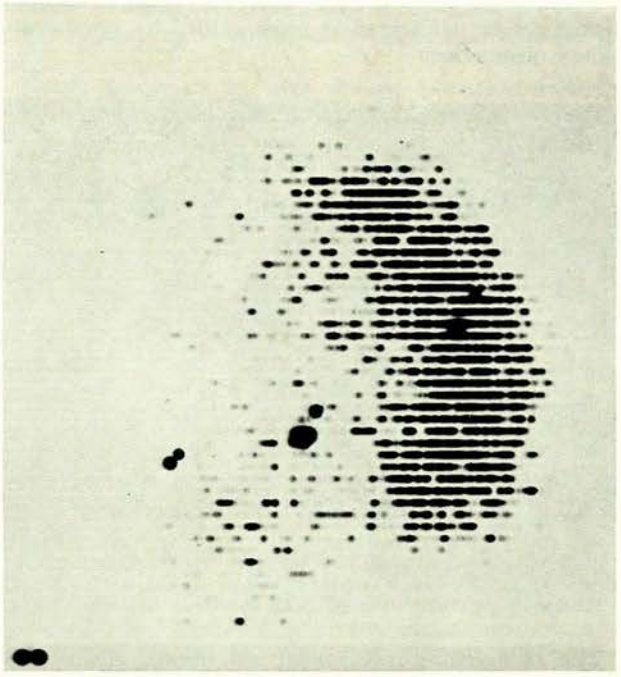

Fig. 5. Lateral scan (case 2) showing the defect to be situated posteriorly.

hepatitis and disease other than biliary atresia, uptake of the isotope by the liver was retarded, but intestinal excretion occurred in all these patients, although usually at a slower rate than normal. In a few instances, however, the results of the radio-iodine rose bengal method and those of liver biopsy were conflicting and it was extremely difficult to come to a definite diagnosis in one or two of these cases. Some of the difficulties encountered are illustrated by the following examples:

Case 3. P.H., a 19-month-old Bantu infant, was brought to hospital because the mother noticed blood in his stools and redness of the left eye, which began 3 days previously. On examination he was found to be jaundiced. He also had scaling of the skin, nasal discharge, subconjunctival haemorrhage of the left eye and hepatosplenomegaly.

The urine showed bilirubin +, but no inclusion bodies were noted in epithelial cells in the urinary sediment. His haemoglobin concentration was 4 G/100 ml., and his red blood cell count was 1,630,000/cu.mm. Serologic tests for syphilis were positive (Kolmer complement-fixation test +; Price precipitation test 640 units). Total serum proteins were 4·6 G/100 ml., serum albumin comprising 46·5% of the total protein content and serum globulin 53·5% (α_1 8·3%, α_2 9·5%, β 22·4%, γ 13·3%). Other results were as follows: Thymol turbidity 14, thymol flocculation +++, total serum bilirubin 10·0 mg./100 ml., conjugated serum bilirubin 9·6 mg./100 ml., alkaline phosphatase 57 KA units, SGOT 103 Cabaud units, SGPT 94 Cabaud units, LDH 353 μmol. DPNH/100 ml./30 minutes.

Needle biopsy of the liver (Fig. 6) showed marked bile stasis, large bile thrombi in the bile ducts and in the portal tracts, and proliferation of the bile ducts. A few giant

multinucleated cells were also observed. These histological changes were interpreted as those caused by extrahepatic biliary obstruction.

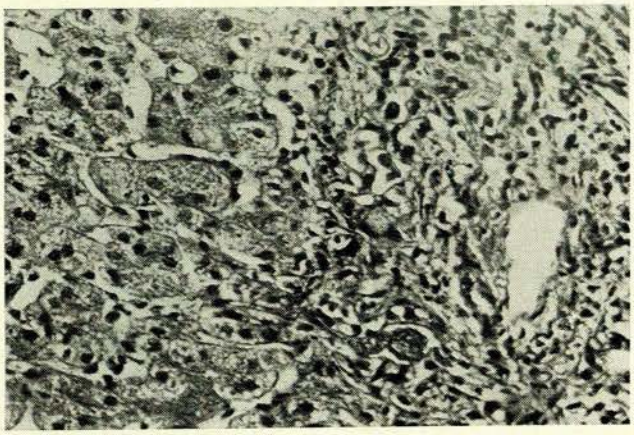

Fig. 6. Histological features of liver (case 3). Although the main changes (bile stasis, bile thrombi and proliferation of the bile ducts) were compatible with a diagnosis of biliary atresia, the patient suffered from syphilitic hepatitis.

The radio-iodine rose bengal test showed efficient uptake and concentration of the dye by the liver and good intestinal excretion occurred.

A course of penicillin was given and the patient improved. The serum bilirubin decreased rapidly and the other liver-function tests improved gradually. The liver decreased in size and the infant was discharged, apparently cured, about 4 weeks after admission.

Although the histological features of the liver were indicative of biliary atresia, the patient undoubtedly had syphilitic hepatitis. If the radio-iodine rose bengal test had not been done the patient might have been subjected to unnecessary surgical exploration.

Case 4. K.H., a 2-month-old White infant, had been jaundiced since soon after birth. On the day before admission he became irritable and feverish. The main findings were jaundice, an enlarged, hard liver and a palpable spleen.

The urine showed bilirubin +, no reducing substances and no inclusion bodies in epithelial cells of urinary sediment. The haemoglobin concentration was 9·2 G/100 ml., the red blood cell count 3,030.000/cu. mm., and the white blood cell count was 13,000/cu.mm. Serological tests for syphilis and toxoplasmosis were negative.

Liver-function tests gave the following results: prothrombin time 32% of normal, total serum protein 5·2 G/100 ml., serum albumin 42·6%, serum globulin 57·4% (α_1 7·4%, α_2 14·8%, β 18·8%, γ 16·4%), thymol turbidity 1, thymol flocculation 0, total serum bilirubin 5 mg./100 ml., conjugated serum bilirubin 4 mg./100 ml., alkaline phosphatase 48 KA units, SGOT 46 Cabaud units, SGPT 85 Cabaud units, LDH 246 μmol. DPNH/100 ml./30 min.

Serial scans with the rose bengal test (Fig. 7) carried out over a period of 2 days failed to show any activity over the intestinal area, and extrahepatic biliary atresia was regarded as the likely diagnosis. However, when the scan was repeated 7 days after the injection a small area of radioactivity was detected over the left iliac fossa (Fig. 7).

Unfortunately permission to perform liver biopsy could not be obtained from the parents. About 3 weeks after admission the serum bilirubin began to decrease. A control liver scan was carried out about 24 days after the initial investigation. At this stage good intestinal excretion of the isotope occurred (Fig. 8). The infant was discharged about 7 weeks after admission, and when seen again a few months later he was apparently quite well.

This patient illustrated that absence of radioactivity over the intestinal area may occur in infants with liver disease other than biliary atresia. A diagnosis of 'neonatal hepatitis' was made on clinical grounds. Although the first radio-iodine rose bengal test showed no detectable excretion of the isotope during the first 2 days, an area of radioactivity was found over the left iliac fossa on the 7th day. The second radio-iodine test and clinical course ruled out a diagnosis of biliary atresia.

Case 5. B M., a Bantu infant of 8 months, was brought to hospital because of diarrhoea and coughing which began 5 days previously. The infant was markedly jaundiced, the spleen was palpable and a hard liver was felt 2 cm.

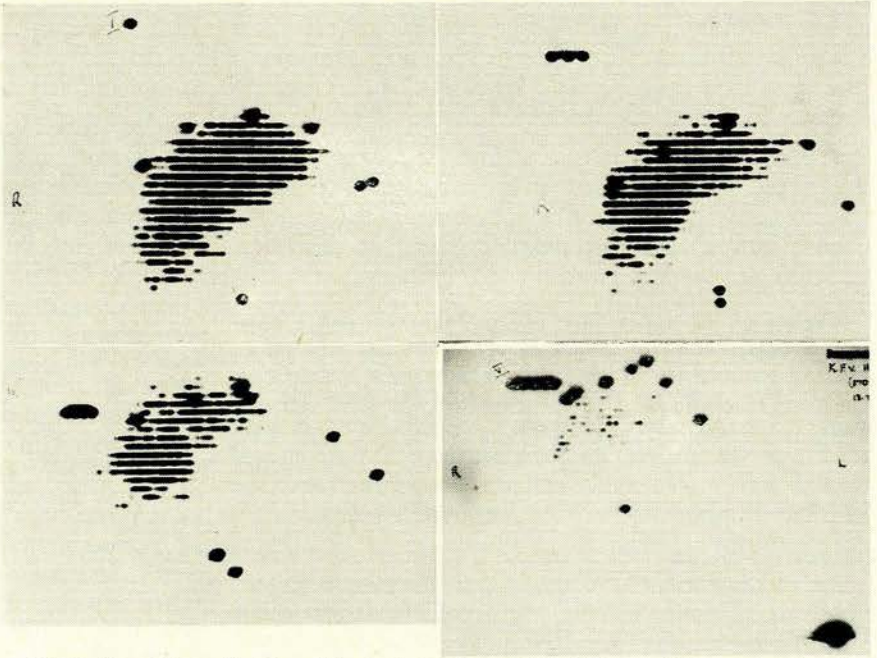

Fig. 7. Scans (case 4) taken 40 minutes (*above, left*), 25 hours (*above, right*) and 47·35 hours (*below, left*) after the injection of radio-iodinated rose bengal fail to show any intestinal excretion of the isotope. The scan taken 7 days after injection (*below, right*) shows an area of activity over the left iliac fossa.

below the costal margin.

The urine showed bilirubin +, urobilin +, no reducing substances were present and there were no inclusion bodies in epithelial cells in the urinary sediment. The faeces contained stercobilinogen 48·5 mg./100 ml. The haemoglobin concentration was 7·9 G/100 ml., the red blood count was 3,160,000/cu.mm. and serological tests for syphilis and toxoplasmosis were negative. Liver-function tests showed prothrombin time to be 51% of normal, total serum proteins 6 G/100 ml., serum albumin 34·4%, serum globulin 65·6% (α_1 7·3%, α_2 10·8%, β 11·7%, γ 35·8%), thymol turbidity 13, thymol flocculation ++++, total serum bilirubin 7·1 mg./100 ml., conjugated serum bilirubin 7·1 mg./100 ml., SGOT 74 Cabaud units, SGPT 23 Cabaud units, LDH 246 μmol. DPNH/100 ml./30 min. and alkaline phosphatase 17 KA units.

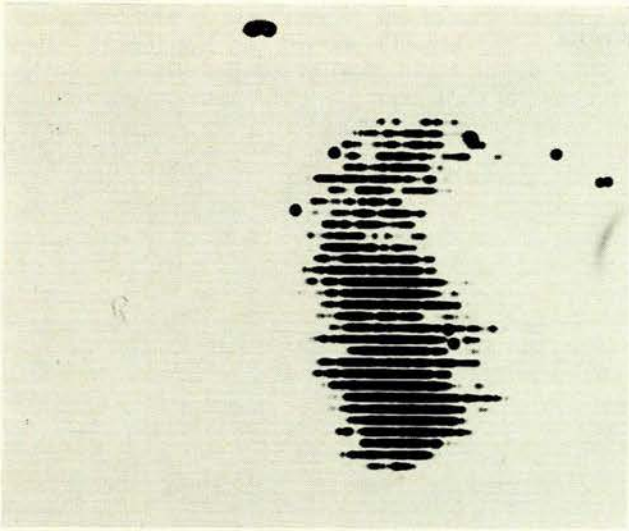

Fig. 8. Control liver scan (case 4) carried out 24 days after the initial investigation, showing good intestinal excretion of the isotope.

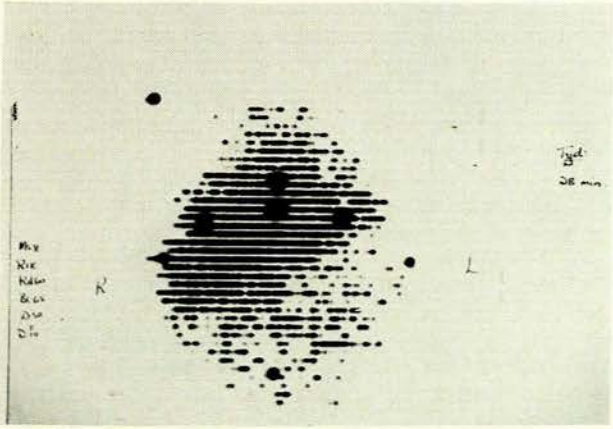

Fig. 9. Liver scan (case 5) carried out 28 minutes after injection of the isotope, showing definite activity over the intestinal area. Intestinal excretion, although delayed, was also noted in all subsequent scans carried out over a period of 4 days.

Serial scanning over a period of 4 days showed definite, although retarded, intestinal excretion of the isotope (Fig. 9).

Needle biopsy of the liver showed bile stasis and bile thrombi in the bile ducts and canaliculi, marked proliferation of the small bile ducts and periportal fibrosis (Fig. 10).

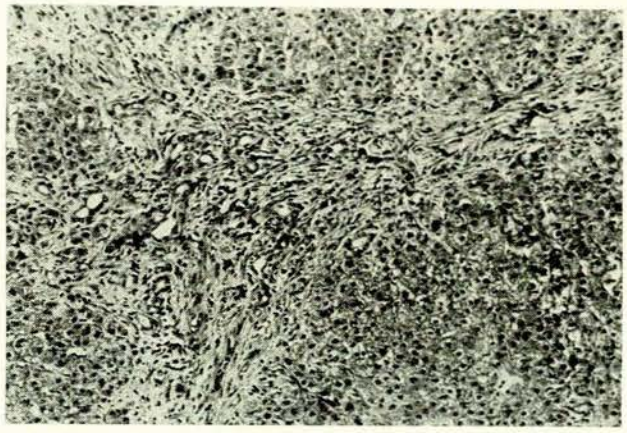

Fig. 10. Histological features of the liver of case 5, showing typical changes of extrahepatic obstruction, i.e. bile stasis, bile thrombi, proliferation of the small bile ducts and periportal fibrosis.

These changes were regarded as typical of extrahepatic biliary obstruction.

Because of the biopsy findings laparotomy was deemed advisable, although the liver scan was not suggestive of biliary atresia. At operation a rudimentary gallbladder was found. The common bile duct could not be found and no bile ducts were found in the porta hepatis. Cholangiography was attempted, but failed.

A diagnosis of extrahepatic biliary atresia was made, which was supported by the wedge-shaped biopsy specimen of the liver taken at operation, which showed histological changes similar to those seen in the specimen taken by needle puncture.

The radio-iodine rose bengal test was repeated, and the scan again showed delayed but definite activity over the intestinal region. For a period of 5 days after the injection of the isotope all the diapers of the infant were collected. Those containing faeces and urine were deposited in one container and those containing urine alone in another. The sets of diapers were measured for radioactivity in the whole-body counter. Radioactivity of the diapers containing both faeces and urine was approximately 1½ times greater than that of the diapers containing urine alone.

The infant has now been in hospital for 3 months and no improvement has occurred. The total serum bilirubin level has increased to 16 mg./100 ml. and the stools remain clay-coloured.

This patient presents a difficult diagnostic problem. The biopsy and operative findings favour a diagnosis of extrahepatic biliary atresia. On the other hand, the 2 series of radio-iodine rose bengal tests suggest that intestinal excretion of the isotope, although very much retarded, did occur. This is supported by the higher radioactivity found in the diapers containing faeces than in those containing

urine alone. It is difficult to reconcile the results of the isotope studies with the operative and biopsy findings. There are, however, possible explanations which will be discussed presently.

DISCUSSION

As can be deduced from the results of the present study, and in particular from the illustrative case reports, it is obvious that the radio-iodine rose bengal test can be most useful, but that it also has its limitations.

The value of the test as a diagnostic aid in parenchymatous liver disease is well established.[2,3,5,16] The most common liver disease in childhood is infectious hepatitis (Tables I - IV). Liver scans of patients with this disease may have a characteristic patchy pattern.[2] However, the diagnosis of infectious hepatitis seldom presents any difficulty and liver scanning, therefore, is seldom indicated. A similar pattern may be seen in the scans of patients with cirrhosis and the test can be a useful aid in the diagnosis of this disease (Fig. 1). In contrast with infectious hepatitis, scans of infants with 'neonatal hepatitis' usually do not have a patchy appearance and the test may give an indication as to whether or not cirrhosis has supervened. This pattern is non-specific and is only an indication of diffuse parenchymatous involvement of the liver.[2] Occasionally it may be difficult to distinguish the pattern of hepatitis and cirrhosis from that caused by metastases.[2] Liver scanning alone, therefore, is inadequate in the diagnosis of cirrhosis, but must be confirmed by other methods, particularly by biopsy of the liver.

The value of the radio-iodine rose bengal method as an aid in the diagnosis of other parenchymatous lesions is illustrated by case reports 1 and 2. Once again liver scanning is usually inadequate in establishing the cause of these lesions and, as a rule, liver biopsy is necessary to confirm the diagnosis except, for instance, in those patients where the presence of malignancy (e.g. neuroblastoma and Wilms's tumour) has been established in other organs.

The most useful application of the radio-iodine rose bengal method in childhood is as an aid in differentiating biliary atresia in infants from intrinsic liver disease causing obstructive jaundice.[7-9] This problem is very common. Forty-three of the 50 Bantu infants and 7 of the 9 White infants (Tables I and II) presented with obstructive jaundice.

With one exception, all the scans of all infants with obstructive jaundice due to intrinsic liver disease showed intestinal excretion of the isotope, although often delayed. The exception was the infant with 'neonatal hepatitis' (case 4) in whom no radioactivity could be detected over the intestinal region during the first 48 hours following the injection. At that stage a diagnosis of extrahepatic biliary atresia was considered likely. However, a scan done 7 days after injection showed a distinct area of radioactivity in the left iliac fossa. This finding indicates that if no activity occurs over the intestinal area during the first 48 hours, scans should nevertheless be repeated daily for at least another 5 days. Previously a scanning period of 24 hours was regarded as sufficient.[7] It is obvious that such a short period is inadequate.

Sharp *et al.*[8] compared the method of external surface counting with the method of measuring radioactivity in urine-free stools collected over a 48-hour period. They found that when faecal excretion was less than 15% of the dose given, insignificant counts occurred over the intestinal region of infants with 'neonatal hepatitis', and concluded that surface counting alone could not distinguish these infants from patients with biliary atresia. It may well be, however, that the study period of 48 hours was too short.

There is difference of opinion as to whether biliary atresia and 'neonatal hepatitis' can be differentiated by means of liver biopsy alone.[10-13,15] Although some pathologists have confidence in their ability to differentiate between the 2 entities, it has now become clear that such a distinction is often quite impossible.[12] It has recently been shown in a large series of 132 patients that extensive overlapping of the histological features regarded as typical of 'neonatal hepatitis' and extrahepatic biliary atresia can occur.[12] One of our patients (case 3) who undoubtedly had syphilitic hepatitis showed the histological features usually regarded as typical of extrahepatic biliary atresia (Fig. 6). The case report of this infant, who showed good intestinal excretion of radio-iodine rose bengal, illustrates that liver biopsy cannot be relied upon to give the correct answer in every case.

It is difficult to explain the conflicting findings obtained in one of our patients (case 5). Both the biopsy (needle and wedge specimens) and the operative findings were suggestive of extrahepatic biliary atresia. The radio-iodine rose bengal test, however, performed on 2 occasions, seemed to indicate that intestinal excretion of the isotope, although retarded, did occur (Fig. 9). Sharp *et al.*[8] encountered occasional bursts of inexplicable activity over the left iliac fossa in cases of biliary atresia. It is well known that in patients with diseases causing retarded excretion of rose bengal, the radio-iodine is excreted by the kidneys.[2,3,5] The right kidney is often obscured by the liver, but it may be difficult to make a distinction between activity in the left kidney and small bowel.[2] It is usually possible, however, to identify the kidney by means of its position and appearance, and by doing serial or lateral scans. Serial scans were done in the case of this infant, and excluded the possibility of renal radioactivity. In the presence of poor excretion, radioactivity may also occur over the region of the spleen. In the patient under discussion, however, activity was not limited to this area.

It seems more likely that intestinal excretion of the isotope did occur, especially as the diaper containing faeces showed considerably more radioactivity than those containing urine alone. As mentioned before, the operative diagnosis of extrahepatic biliary atresia is often inaccurate.[14,15] Spontaneous recoveries have been reported in infants whose lesions were regarded as impossible to correct at operation.[15] Another possibility is that the lesion was an example of 'biliary hypoplasia'.[5,17] In this condition the bile ducts are not atretic, but are so poorly developed that complete or incomplete biliary obstruction may ensue. The histological features of the liver are identical with those which occur in biliary atresia.[17]

Although the possibility exists that the radio-iodine rose bengal test is not invariably accurate, it nevertheless appears to be a valuable diagnostic test in obstructive

jaundice in infancy. There is no doubt that liver biopsy alone cannot be relied upon in differentiating biliary atresia and 'neonatal hepatitis'. The chances of making a correct diagnosis, however, would be enhanced if both needle biopsy and the rose bengal test were performed in cases where the diagnosis was in doubt.

SUMMARY

A total of 67 radio-iodine rose bengal tests were carried out on infants and children with liver disease and obstructive jaundice. The method was found to be useful in aiding the diagnosis of parenchymatous lesions of the liver such as cirrhosis, malignancy and abscess.

Although apparently not infallible, the most useful application of the test was in distinguishing between extrahepatic biliary atresia and intrinsic liver disease causing obstructive jaundice.

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