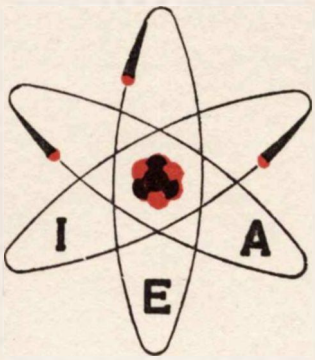

FECAL EXCRETION OF ROSE BENGAL I^{131} IN THE DIAGNOSIS OF OBSTRUCTIVE JAUNDICE IN INFANCY WITH SPECIAL REFERENCE TO BILIARY ATRESIA

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SUMMARY

Some forms of neonatal jaundice, particularly those due to neonatal hepatitis and to atresy of the extra-hepatic bile ducts may present incharacteristic clinical and laboratory features, making differential diagnosis difficult. In search of a discriminative and simple test 48 h cumulative fecal excretion of Rose bengal ^{131}I was tried. Study was done on 27 newborn and infants aged between 11 days and 10 months, who were distributed in 3 clinical groups: I - normal controls (5); II - nonatretic jaundice (6) and III - atretic jaundice as confirmed by laparotomy (16). Technical details that ensure the complete and separated collection of the feces and that simplify the measurement of the radioactivity are given. The mean values of the 48 h cumulative fecal excretion of ^{131}IRB were 49% of the delivered dose in group I, 13,18% in group II and 3.36% in group III. In view of the spread of the results excretion values equal to or less than 5.8% of the dose strongly suggest the existency of atresy of the bile dutcts.

Some forms of neonatal jaundice, particularly those due to neonatal hepatitis and to atresia of the extrahepatic bile ducts, may be atypical in their clinical picture and laboratory data, making their diagnosis difficult. The conventional clinical tests, and even the most recent enzyme determinations, contribute in some degree, but not in a decisive way, to differential diagnosis.[1]

Many investigators have tried to obtain more reliable information by studying the hepatic clearance of a labeled dye - Rose bengal I^{131} (RB I^{131})- from the blood stream and its excretion into the bowel.[2-9] Most of them agree that a low percentual fecal excretion of Rose bengal I^{131} (FERB I^{131}) is indicative of biliary atresia; however there is a considerable variation in the presented values, which may be attributed mainly to differences[3-6,8] in technical procedures.

The inconsistency of the results published up to now led us to study the RB I^{131} excretion test according to the following program.

MATERIAL AND METHODS

The study was done on 27 children including newborn and infants.† According to the final diagnosis, they were distributed in three groups: I. normal controls - five patients; II. nonatresic jaundice - six patients: five cases of intrahepatic colestasis of different etiology and one case of hypoplasia of the external bile ducts; III. atretic jaundice (confirmed by exploratory laparotomy) - 16 patients. All except two infants were submitted to only one test; in two infants the test was repeated.

Once a complete separation between urine and stools had been ensured, it was assumed

† Patients from the Pediatrics Department (Prof. E. Marcondes) and Pediatric Surgery Division.

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that the percentage of a given dose of RB I^{131} appearing in the stools would actually represent the fraction reaching the bowel through the permeable bile ducts. Our preliminary observations allowed us to conclude that the urinary excretion of the radioisotope varies in an irregular way and is not a reliable measurement. For that reason RB I^{131} determination was limited to fecal excretion.

Three days prior to the dose administration, each patient received two drops of a Lugol's solution to block thyroid I^{131} uptake. RB I^{131} was administered intravenously in the approximate dosage of 1 uCi per kilo body weight.†

In order to adequately separate urine from stools, a special type of plastic bag was used. This bag presents an opening (circular for boys and elliptical for girls) surrounded by an adhesive tape which can be well fitted to the perineal region. By the time of the test, the children wore ordinary diapers. After each bowel movement, the diapers containing the stools were taken off and transferred into cans of 1 liter capacity. No manipulation of the excreta was necessary. Thus, losses of material or eventual radioactive contamination was practically excluded. The stool collection was carried for 48 hours from the moment the dose was administered. In order to make the test less troublesome for the child and to reduce the time of possible urine contamination, we decided to shorten the collection period from 72 hours[3,8] to 48 hours.

For each case a reference standard was prepared by diluting an equal dose to that administered, to an appropriate volume with potassium iodide and nonradioactive Rose bengal as carriers. A known fraction of that solution was then distributed at random over sawdust contained in a can, identical to the ones used for the stool samples. Both the dose for the patient and that for the reference standard were measured with a syringe that yields a fixed volume; reproducible within an error of less than 1%.

To determine fecal and reference standard radioactivity, all cans were counted in such a manner (geometric conditions) that the irregular distribution of radioactivity in the carrying material would not contribute appreciably to the counting rate. For this purpose, a Tobor‡ N-C counting device was used which basically consists of two 3 x 3 in. crystal scintillation heads in diametric opposition with their pulses added, then analyzed and registered on a scaler. The arrangement is heavily shielded. Counts were done on the 364 keV photopeak. Counting times were protracted sufficiently to reduce statistical error to less than 1.5%. The total fecal radioactivity was expressed as a percentage of the administered dose. The total percentual efficiency of the detector system was determined and found to be around 10% of the theoretical one. The minimal amount of radioactivity that could be detected was 0.5nCi for 10 minutes counting time with a statistical error of 1%.

For the purpose of statistical analysis, the percentage values were substituted by the arco sin of the square root of the percentage figures. This transformation will more heavily weight the small percentages which have small variance.

The reproduction of the percentage values permitted us to arrive at the figure for an estimate of the upper limit of the percentage for the atretic group as well the lower limit for the nonatresic jaundiced group ($p < 0.05$).[9]

RESULTS

The results of the 48-hour FERB-I^{131}, expressed as the percentage of the administered dose, as well as data concerning identification, sex, age and diagnosis of each patient are listed on Table I.

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† RB I^{131} was furnished by the Radiofarmacy Laboratories of the Institute of Atomic Energy, São Paulo. Free iodine content was systematically checked and never exceeded 2%.

‡ Tobor Model 4350. Nuclear Chicago Corporation, 2000 Nuclear Drive, Des Plaines, Illinois.

TABLE I

48-Hour Percentage Fecal Excretion of Rose Bengal I^{131} in 27 Newborn and Infants (29 Tests)

Number of Order	Name	Sex	Age (months)	Fecal Excretion (48 H-% D)	Diagnosis
1	TZ	M	3	37	Control
2	WS	M	11 d.	22.1	Control
3	JCV	M	3	44.4	Control
4	MG	M	1	71.5	Control
5	IS	M	1.5	70	Control
6	DD	M	2	9.5	Neonatal hepatitis
7	CVS	M	3	22	Neonatal hepatitis
8	CPS	F	3	10.5	Neonatal hepatitis
9	HK	M	3	11.3	Neonatal hepatitis
10	HNK	M	3	16.9	Neonatal hepatitis
11	MEAP	M	4	6.9	Hypoplasia of bile ducts
12	MEAP	M	6	17.3	Hypoplasia of bile ducts
13	RR	M	7	2.6	Biliary atresia
14	RTS	F	10	4	Biliary atresia
15	SS	F	1	4.7	Biliary atresia
16	JLFM	M	6	4.7	Biliary atresia
17	ACO	M	3	0.9	Biliary atresia
18	ECM	F	6	6.6	Biliary atresia
19	ORO	M	4	1.6	Biliary atresia
20	PHBF	M	2	3.3	Biliary atresia
21	GJ	M	3.5	3.2	Biliary atresia
22	WMC	F	4	4.1	Biliary atresia
23	RMB	F	2	0.6	Biliary atresia
24	WFS	M	4	2.9	Biliary atresia
25	NN	M	4	3.6	Biliary atresia
26	NMS	M	4	2.3	Biliary atresia
27	EA	M	4	3.7	Biliary atresia
28	EF	M	6	3	Biliary atresia
29	EF	M	6	5.3	Biliary atresia

Table II reproduces the mean value and the range of the FERB I^{131} for the three groups studied, showing that there was practically no overlapping.

Table III gives the mean and standard deviation for the calculated arco sin of the square root of the percentage values, as well as the result of the significance test for the differences between the means. As can be seen in the third column, these differences were statiscally significant ($p < 0.05$).

According to the obtained data, the estimated upper limit value ($p < 0.05$) is 5.8%. The lower limit estimated value of the nonatresic jaundice group is 6.1%.

TABLE II

48-Hour Fecal Excretion of Rose Bengal I^{131}: Mean Value and Range for the Three Clinical Groups Studied

Groups	Number of Cases	Mean Value % of Dose	Range % of Dose
Group I - Controls	n = 5	49.00	22.1 - 71.5
Group II - Nonatresic jaundices	n = 7	13.18	6.9 - 22.0
Group III - Biliary atresia	n = 17	3.36	0.6 - 6.6

TABLE III

Statistical Analysis: Mean, Standard Deviation, and Significance Test of the Arco Sin of the Square Roots of the Percentage Values for the Three Clinical Groups Studied

Groups	Arco Sin $\sqrt{\%}$ Mean	Standard Deviation	"t" Test
Group I - Controls	44.36	12.80	I x II $p < 0.05$
Group II - Nonatresic jaundices	21.22	4.49	II x III $p < 0.05$
Group III - Biliary atresia	10.25	2.69	III x I $p < 0.05$

COMMENTS

The authors generally agree that the FERB-I^{131} test yields valuable information for the differential diagnosis of neonatal jaundice. However, the ranges reported in literature for the different clinical entities often present large overlapping areas. The general opinion is that its current performance is rather difficult because of the problems encountered in obtaining a separation between urine and fecal excretion in the child.[4,6]

The results obtained in the present series could be separated into three groups with significant statistical difference and each group could be related to a well-defined clinical situation (Table III).

It is our feeling that the technical efforts described above, mainly the manner of separating urine from stools, and the technique used for the collecting and counting of the samples, accounted for our consistent results. Moreover, by this simpler methodology and the reduction of the collecting period, the test became easily feasible for routine purposes.

The nearness of the upper limit estimated value of the atresia group (5.8%) and the lower limit estimated value of the hepatitis group (6.1%), obtained by statistical calculations, does not invalidate the practical value of the test.

There is a range of values, close to 5.8 to 6.1% for which we thought better to repeat the test.

The sensitivity and screening power of the test can be further appreciated through the two cases presented below.

CASE REPORTS

E.F. (tests 28 and 29, Table I), a 6-month-old infant with previous clinical and histo-pathologic diagnosis of intra-hepatic colestasis. The result of a first FERB I^{131} test (3.05%) suggested total obstruction of the bile ducts. In view of the other laboratory data and the clinical picture pointing to hepatitis, the test was repeated after 6 days: the 48 hour FERB I^{131} (5.3%) persisted within the atresia range. Atresia was later confirmed by laparotomy.

M.E.A.P. (tests 11 and 12, Table I) a 4-month-old infant whose clinical and laboratory results, including a FERB I^{131} of 6.9% suggested the diagnosis of possible biliary atresia, although this was a relatively high percentage figure, when compared with the other proved cases of biliary atresia (Table I). When this infant was operated on, hypoplasia of the biliary ducts was found and mechanical clearing of the biliary tree was attempted by the surgeon. Because of persisting jaundice after operation, a second test was performed; the result of which was now 17.3% fecal excretion, a value which is consistent with the clinical improvement noted post-operatively.

CONCLUSION

Despite the poor prognosis of biliary atresia, there are a small number of cases in which early surgery will be of real benefit. Every effort must therefore be made to arrive at the correct diagnosis as quickly as possible so as not to delay surgical intervention.

In our experience, FERB I^{131} has been the most reliable diagnostic test; we are convinced that this test must be done in every case in which the differential diagnosis of neonatal or infantile jaundice is not clear.

RESUMO

Algumas formas de icterícia neonatal, particularmente aquelas devidas à hepatite neonatal e à atresia das vias biliares extra-hepáticas, podem apresentar quadros clínicos e laboratoriais atípicos, o que dificulta o diagnóstico diferencial. Objetivando uma prova discriminativa de fácil execução, ensaiou-se a excreção fecal cumulativa de 48 horas de Rosa Bengala ^{131}I. Estudaram-se 27 crianças com idade variando entre 11 dias a 10 meses, distribuidas em três grupos: I - Controles normais (5), II - icterícia não atrésica (6) e III - icterícia devida a atresia de vias biliares (16) confirmada por laparotomia exploradora. Detalhes técnicos que garantem a coleta separada de urina e fezes e que facilitam a determinação da atividade são referidos. Os valores médios de excreção fecal cumulativa de 48 h foram de 49% da dose para o grupo I, 13,18% para o II e 3,36% para o III. Em face da dispersão dos resultados, excreções iguais ou inferiores a 5,8% da dose são altamente sugestivas de atresia das vias biliares.

RÉSUMÉ

Quelques formes d'ictère de la période néonatale, particulièrement celles determinées soit par l'hépatite néonatale soit par l'atrésie des voies biliaires extra-hépatiques, peuvent présenter un tableau clinique et biochimique incaractéristique, ce qui difficulte le diagnostic différentiel. Dans la recherche d'un test à la fois discriminatif et d'éxécution simple, on a éssayé l'excrétion fécale cumulative de 48 heures de la Rose Bengale - ^{131}I. L'étude a été conduite sur 27 nouveaux-nés et nourrissons âgés de 11 jours à 10 mois et qui ont été distribués en trois groupes cliniques: I - sujets témoins (5); II - ictère sans atrésie des voies biliaires (6); III - ictères à cause d'atrésie des voies biliaires confirmée par laparotomie. On réfère les détails techniques qui apportent à un recueil complet et separé des selles et qui simplifient la mesure de la radioactivité. Les valeurs moyennes de l'excrétion fécale cumulative de 48 heures ont été les suivantes: 49% de la dose injectée pour le

groupe I, 13,18% pour le groupe II et 3,36% pour le groupe III. En vue de la dispersion des résultats, des excrétions jusqu'à 5,8% de la dose sont vivement suggestives d'une atrésie des voies biliaires.

REFERENCES

1. Holder, T.M.: Atresia of the extrahepatic bile duct. Amer. J. Surg., 107: 458, 1964.
2. Becker, F.B., and Hoeffler, D.F.: The radioactive rose bengal test. Its value in the diagnosis of extra hepatic biliary atresia in young infants. Clin. Pediat., 3:714, 1964.
3. Brent, R.L., and Geppert, L.J.: The use of radioactive rose bengal in the evaluation of infantile jaundice. Amer. J.Dis. Child., 98: 720, 1959.
4. Desbuquois, B., Troix, P., and Alagille, D.: Étude de l'excrétion fecale et urinaire du rose bengal marqué par l'iode radioactif an cours des ictères déstructifs du nouveau-né et du nourrisson. Arch. Franç. Pediat., 25: 379, 1968.
5. Geppert, L.I., and Brent, R.L.: Radioactive rose bengal: An aid in the differential diagnosis of the jaundiced infant. Amer. J.Dis. Child., 94: 544, 1957.
6. Ghadimi, H., and Sass-Kortsak, A.: Evaluation of the radioactive rose bengal test for the differential diagnosis of obstructive jaundice in infancy. New Eng. J. Med., 265: 35l, 1961.
7. Marzano, E., and Turla, B.: Il comportamento della prova con rosa bengala radioiodato nella diagnostica di alcune affezione epatiche dell'étà pediatrica: atresia delle vie biliari extrapatiche colostasi intraepatiche, epatiti. Acta Paediat. Lat., 18: 110, 1965.
8. Schmidt, B.J.: A prova da rosa bengala radioativa (RB I^{131}) no diagnóstico diferencial das icterícias obstructivas do lactente. Tese - Escola Paulista de Medicina - 1966.
9. Snedecor, G.W.: Statistical Methods. Ames, Iowa: The Iowa State College Press. p. 316, 1956.
10. White, W.E., Welch, J.S., Darrow, D.C., and Holder, T.M.: Pediatric application of the radioiodine (I^{131}) rose bengal method in hepatic and biliary system disease. PEDIATRICS, 32:239, 1963.