LIVER FUNCTION IN NEWBORN INFANTS

WITH SPECIAL REFERENCE TO EXCRETION OF BROMSULPHALEIN

BY

SIMON YUDKIN, Ph.D., M.B., M.R.C.P., D.C.H.

Nuffield Fellow in Child Health

and

SYDNEY S. GELLIS, A.B., M.D.

with the technical assistance of

FRANCES LAPPEN, B.S.

(From the Department of Paediatrics, Harvard Medical School, the Children's Medical Centre and the Boston Lying-in Hospital, Boston, Massachusetts, U.S.A.)*

The theory that neonatal jaundice is produced mainly by haemolysis of excess red cells present at birth has been strongly criticized in recent years (Weech, 1947; Findlay, 1946) and increasing emphasis has been placed on the role of the liver in the genesis of this form of jaundice (Weech; Findlay; Mollison, 1948). Direct evidence that the liver in newborn infants is unable to function adequately is, however, scarce and somewhat contradictory (see Weech for references). It was felt that investigation of the functions of the liver in newborn infants with special reference to its excretory capacity might help to clarify the position.

Material and Methods

BROMSULPHALEIN EXCRETION TESTS (Rosenthal and White, 1925; Mateer et al., 1943). These were carried out on normal full-term infants at the Boston Lying-in Hospital. The infants were between 8 and 216 hours old. Single determinations were made on the majority of the infants but a few had two tests and two infants had three tests. The test dose of 5 mg. of dye per kg. body weight was injected into a scalp vein and a sample of 0.2 to 0.3 ml. of blood was taken forty-five minutes later from a stab wound in the heel into a glass tube of 2 mm. internal diameter and 10 cm. long; small rubber caps were used to close the ends of the tube. The serum was removed after centrifuging and the bromsulphalein level was determined by the method described by Gaebler (1945) but the microcolorimeter attachment of the Evelyn photoelectric colorimeter was used instead of the macrocolorimeter, the volumes being adjusted accordingly (0.1 ml. serum, 0.5 ml. water, and 0.6 ml. of

0.1N NaOH). It was thus possible to make measurements on capillary blood, a useful consideration in the newborn infant. Preliminary measurements on capillary blood and venous blood taken simultaneously at varying intervals after injection of dye showed that the results were in agreement so long as the blood from the heel was flowing freely when collected.

Percentage retention of the dye was calculated according to the method of Gaebler (10 mg. per 100 ml. serum = 100 per cent. retention).

OTHER TESTS. In addition, samples of about 5 ml. of blood were taken from the internal jugular vein of thirty-two other infants from one to seven days old and the following tests carried out on the serum: (a) thymol turbidity and thymol flocculation (Maclagan, 1944); (b) cephalin cholesterol flocculation (Hanger, 1939; Neefe and Reinhold, 1944); (c) colloidal gold (Maclagan, 1946).

'One minute' and total serum bilirubin levels were measured in most of the infants in both groups using the photoelectric colorimeter (Malloy and Evelyn, 1937; Watson and Hoffbauer, 1947).

Results

Bromsulphalein excretion tests. The figure shows the results for all the eighty-three tests that were done. It is apparent that during the first four days of life most of the babies showed much more retention (i.e. less excretion) than did the babies of four to nine days of age. The results are arranged in the table to show the number of babies in each of three different age groups having retention of dye above and below the mean retention for the whole group. Calculation of the χ^2 value shows that this distribution of results is most unlikely to have arisen by chance ($p < 0.01$).

It should be noted that even the babies of four to nine days showed more retention than that

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regarded as normal for healthy adults using the same dosage of dye (Mateer et al., 1947).

The babies who had more than one test showed results in essential agreement with those already given. Of those that had two tests (on the first and third day of life), none showed more retention on the second test and some showed less. The two babies who had tests on the first, third, and seventh day showed about 14 per cent. retention on the first and third day and about 6 per cent. retention on the seventh day.

There was no correlation between the results of the bromsulphalein tests and the levels of serum bilirubin. It would have been of interest to measure bromsulphalein retention on the first day of life of

showed poor excretion of injected bilirubin in the first few days of life with subsequent improved excretion.

The results of the bromsulphalein tests here recorded are in general agreement with those of Herlitz (1927) who found poor excretion in the youngest infants and no correlation with the presence or absence of jaundice; the excretion was better in older infants. They apparently differ widely from the results reported by Salmon and Richman (1943), but the curve of bromsulphalein excretion given in their report is a composite one from the results in infants of various ages in the newborn period and it is impossible to determine from their data how many of the readings taken

a large number of infants and to determine whether this was related to the height of the subsequent rise in bilirubin levels, but this was not possible at the time the experiment was conducted.

There was no correlation between the retention of dye and the weight of the babies.

Other liver function tests. None of the other tests showed any abnormal results whatsoever.

Discussion

Mollison (1948) showed that the infant's red cells are destroyed after birth about twice as quickly as are red cells obtained from adults but that this of itself would not account for all the rise in serum bilirubin level in the newborn period unless the liver were unable to excrete all the extra load of bilirubin thus produced. He found also that appreciable amounts of bromsulphalein persisted in the serum two hours after injection into infants soon after birth. Fashena (quoted by Weech, 1947), using a method of calculation devised by Weech et al. (1941),

twenty minutes or more after the dye was injected were made on infants during the first two or three days of life.

TABLE
RESULTS OF BROMSULPHALEIN EXCRETION
TESTS IN NEWBORN INFANTS

Age of infants (hours)	Bromsulphalein retention at 45 mins.		
	Above mean	Below mean	No. of infants tested
	Number of infants		
8-48	16	5	21
49-96	12	16	28
97-216	6	28	34
Total	34	49	83

Mean bromsulphalein retention (all tests) 10.2 per cent.
 $\chi^2 = 20.6$ (value for $p = 0.0192$)

Dragstedt and Mills (1936) have criticized the use of the bromsulphalein excretion tests in jaundiced subjects on the grounds that the raised serum bilirubin of itself interferes with the excretion of dye. While this factor may have accounted for some of the high retention levels found on the third and fourth days it could not account for the even higher levels on the first and second days when the bilirubin levels were lower.

The fact that even the babies of four to nine days showed retention greater than that reported for adults suggests that there is a continuing difficulty in excretion. Herlitz (1926) found delayed excretion up to the age of five months.

It is important to note that the function here concerned is that of excretion and that our results give no evidence of poor function in other respects. We were unable to confirm the positive findings with the cephalin cholesterol flocculation tests reported by Salmon and Richman. Thymol turbidity and flocculation tests and colloidal gold tests have not been carried out previously on newborn infants so far as we are aware.

We can at present only speculate as to the possible cause or causes of the retention of dye. It may be related to circulatory adjustments taking place in the first few days after birth, since it is known that circulatory factors are of importance in the excretion of the dye (Blumberg and Schloss, 1947). In view of the negative results with the other tests it seems unlikely that the liver cells are damaged. The continued poor excretion of the dye after the first four days suggests that the liver may be 'immature' as regards its excretory function but the rapid improvement in the first few days makes it appear likely that some other factor is also involved.

Summary

Eighty-three bromsulphalein excretion tests have been carried out on full-term infants from 8 to 216 hours old. The younger infants showed greater retention of dye than did infants over four days of age: the results are statistically highly significant. The infants over four days old showed greater

retention than that reported for adults given equivalent amounts of dye.

There was no correlation between the amount of dye retention and the level of serum bilirubin or between the amount of dye retention and the weight of the infant.

Thymol turbidity and thymol flocculation tests, cephalin cholesterol flocculation tests, and colloidal gold tests carried out on the serum of thirty-two other infants of the same age range showed no abnormality whatever.

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REFERENCES

- Blumberg, N., and Schloss, E. M. (1947). *Amer. J. med. Sci.*, **213**, 470.
 Dragstedt, C. A., and Mills, M. A. (1936). *Proc. Soc. exp. Biol., N.Y.*, **34**, 467.
 Fashena, G. (1947). Quoted by Weech (1947).
 Findlay, L. (1946). *Arch. Dis. Childh.*, **21**, 195.
 Gaebler, O. H. (1945). *Amer. J. clin. Path.*, **15**, 452.
 Hanger, F. M. (1939). *J. clin. Invest.*, **18**, 261.
 Herlitz, C. W. (1926). *Acta paediatr., Stockh.*, **6**, 214.
 MacLagan, N. F. (1944). *Brit. J. exp. Path.*, **25**, 234.
 — (1946). *Ibid.*, **27**, 369.
 Malloy, H. T., and Evelyn, K. A. (1937). *J. biol. Chem.*, **119**, 481.
 Mateer, J. G., Baltz, J. I., Marion, D. F., and Macmillan, J. M. (1943). *J. Amer. med. Ass.*, **121**, 723.
 —, —, Comanduras, P. D., Steele, H. H., and Brouwer, S. W. (1947). *Gastroenterol.*, **8**, 52.
 Mollison, P. L. (1948). *Lancet*, **1**, 513.
 Neeffe, J. R., and Reinhold, J. G. (1944). *Science*, **100**, 83.
 Rosenthal, S. M., and White, E. C. (1925). *J. Amer. med. Ass.*, **84**, 1112.
 Salmon, G. W., and Richman, E. E. (1943). *J. Pediat.*, **23**, 522.
 Watson, C. J., and Hoffbauer, F. W. (1947). *Ann. int. Med.*, **26**, 813.
 Weech, A. A., Van, D., and Grillo, R. A. (1941). *J. clin. Invest.*, **20**, 323.
 — (1947). *Advances in Pediatrics*. New York, vol. 2, p. 346.