The Extent of 'Shunt' Bilirubin and Erythrocyte Survival in the Newborn Infant Measured by the Administration of [15N]Glycine

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The cause of neonatal jaundice has always been a problem of great interest. At first increased erythrocyte breakdown was thought to be responsible, but lately interest has centred on the enzyme that is responsible for the formation of conjugated bilirubin. Though a diminished activity of glucoronyltransferase has been proved fairly conclusively (Lathe & Walker, 1958), the studies relating to erythrocyte survival are difficult to interpret at this age. This difficulty arises partly from the fact that blood volume is changing and partly from the fact that erythrocyte production is not in a steady state, so that the population does not contain equal numbers of cells of all ages (Mollison, 1961). In both methods commonly used for estimating the life span of erythrocytes, the Ashby technique and labelling with radioactive chromium (Vest & Grieder, 1961), the fate of a mixed population with respect to age is studied. By using [15N]glycine one can follow a more uniform group, as most of the [15N]glycine used for haem formation is incorporated into the erythrocytes that are formed in a few days after the administration. This method also makes it possible to estimate the fraction of bile pigment not derived from mature circulating erythrocytes, i.e. the early labelled or 'shunt' bilirubin (Israels, Yamamoto, Skanderbeg & Zipursky, 1963), and its contribution to the production of bile pigment. The present work was undertaken to determine erythrocyte survival in the newborn infant by incorporation of [15N]glycine into haem and bile pigment, and also to estimate the extent of 'shunt' bilirubin.

A total of 800mg. of [15N]glycine with an isotope content of 95% was given intravenously over a period of 8hr. to each of two newborn infants, S. D. and V. D., 3 and 6 days old and with weights of 3600 and 3350g, respectively. Crystalline haemin was prepared from 2–3ml. samples of venous blood by the method of Labbe & Nishida (1959). Stools were collected for 4–5 days each week. Sterobilin was extracted and crystallized according to the method of Gray (1953). From a suggestion of D. C. Nicholson (personal communication), bilirubin was extracted into NaOH, washed with ether, acidified and extracted into chloroform, from which it was crystallized by addition of methanol. The [15N] content was determined according to the method of Sprinson & Rittenberg (1949) in a Consolidated Nier mass spectrometer. The isotope concentration found in haemin and bile pigments in infant S. D. is shown in Fig. 1.

![Fig. 1. The 15N concentrations in haemin (—) and bilirubin (……) after intravenous infusion of 800mg. of [15N]-glycine into newborn infant S. D. (3days old) are shown.](image-url)
The $^{15}$N concentration in haemin reached a maximum of 0.28 atom percent excess at about the fortieth day, although half that concentration was reached within about 6–7 days. A very similar curve with a maximum of 0.185 atom percent $^{15}$N excess was obtained in the second newborn infant. From the declining part of these curves the average erythrocyte life has been evaluated by the method of Shemin & Rittenberg (1946), taking into account that the labelled erythrocytes are produced over a certain interval of time. It was found to be 85 days in infant S. D. and 91 days in infant V. D.

In Fig. 1 the amount of isotope is given as atoms per cent excess of $^{15}$N in the haem of the circulating erythrocytes. No account has been taken of the fact that in newborn infants total circulating haemoglobin falls by about one-third in the first 6 weeks of life and then starts to increase and reaches the newborn level again at about 4 months. The effect of these changes on the present results is presumably that the initial rise in the curve and the slope of the subsequent decline have been exaggerated. Nevertheless, since the average life span of the erythrocytes is defined as the point of maximal change in the slope of the declining part of the haemin curve, and since total haemoglobin is much the same at the beginning and end of the period, determination of the life span of erythrocytes should not be materially affected.

In infant S. D. only bilirubin was excreted in the stools during the whole duration of the study, whereas in V. D. stercobilin was the chief bile pigment after the eighth week. As in the adult the curve of the $^{15}$N excess of bilirubin–sterobilin shows two peaks. The first peak has a maximum during the second half of the first 4-day collection period (3–4 days after glycine injection). Its isotope concentration is higher than that of the second peak, especially in infant S. D. (0.67 atom percent excess). In infant V. D. it reached 0.27 atom percent excess. By using the assumptions of London, West, Shemin & Rittenberg (1950), it was calculated that at least 21–25% of the bile pigment excreted in the faeces is derived from isotopically labelled precursors that are not the haemoglobin of circulating mature erythrocytes. This early labelled fraction is therefore more than twice as high in the newborn as in normal adults. After the first peak there is a flat part, which lasts from about the twentieth to the seventy-seventh day. The second peak, which is due to destruction of erythrocytes at the end of their life span, reaches a maximum at 97 days in infant S. D. and at 110 days in infant V. D. When allowance is made for the fact that the $^{15}$N concentration in the erythrocytes takes some time to reach a maximum, erythrocyte survival derived from the bilirubin–sterobilin excretion is calculated to be 90–100 days. This agrees reasonably well with the values calculated from the haemin curve. The erythrocyte life span in the full-term newborn infant is therefore shorter than in the adult in which, by the same method, it has been found to be 120–127 days (Shemin & Rittenberg, 1946).