The Postnatal Synthesis of Fetal Hemoglobin. I. Some Studies in Newborn Goats Using Radioactive Fe^{59} and Glycine-2-C^{14}

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ASSUMING an average survival time of the blood cell of the newborn infant of about 80 days,[1, 2] it is clear that the presence of fetal hemoglobin more than 120 days after birth points in the direction of a postnatal synthesis of this fetal pigment. Studying the disappearance of fetal hemoglobin after birth with a direct spectrophotometric alkali denaturation method, some investigators did not find Hb-F after 22 weeks.[3-5] The method used,[6] however, was not accurate below 10 per cent of Hb-F present. With a slightly different but more sensitive method Jonxis and Visser[7] demonstrated the presence of 5 to 10 per cent of Hb-F at 20 weeks after birth. Using their alkali denaturation method, Chernoff and Singer[8] found still percentages of 2.7 up to 27 per cent of alkali resistant hemoglobin in the blood of children of six months and older. The accuracy of this technic, however, especially for the determination of low percentages of fetal Hb, is questionable.[9, 10]

There are many indications that even during adult life, low amounts of an alkali resistant hemoglobin are present in the blood.[11-14] Using alkali denaturation technic Singer and co-workers[11] found 0.5 to 1.7 per cent of such a component, while the amounts established by Künzer[12] are somewhat lower. With a more specific immunologic technic Chernoff[13] made the presence of 0.05 to 0.5 per cent of fetal hemoglobin in the blood of a normal adult more likely. In accordance with these data are the investigations of Huisman, Jonxis and Dozy[14] who have found 0.3 to 0.4 per cent of an alkali resistant hemoglobin with an amino acid composition quite similar to that of pure fetal hemoglobin of the newborn infant. The presence of high amounts of fetal hemoglobin during adult life in Cooley's anemia seems to be certain.[15] The identity of an alkali resistant hemoglobin in other hematologic diseases such as leukemia[11, 13] is not yet established.

These data, although obtained by different quantitative estimation methods are strong indications that a synthesis of fetal hemoglobin occurs after birth. Moreover the reappearance of alkali resistant hemoglobin in the blood of newborn children suffering from erythroblastosis fetalis, after treatment with exchange transfusions, points in the same direction.[16-18]

In this report some experiments are described of the incorporation of radioiron (Fe^{59}) and radioactive carbon (C^{14}) into the fetal and adult hemoglobin of untreated newborn goats and of goats after total blood exchange. In comparing the specific activity of the isolated hemoglobins after injection of Fe^{59} or of

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Submitted Jan. 29, 1957; accepted for publication May 24, 1957.

The authors are much indebted to Dr. K. K. Bossina for his aid in the exchange transfusion experiments, and to Mr. G. A. Broek and Miss J. H. Jans for their technical assistance.

glycine-2-C^{14} it was possible to obtain other indications of a synthesis of fetal hemoglobin after birth.

Material and Methods

Animals and radioactive materials

Three normal newborn goats (bodyweight 3200-5100 Gm.) were used. During the first six weeks they were fed with cow-milk, later with grass and cow-milk. They grew quickly. The average increase in bodyweight was 90 Gm./day. Radioactive materials were injected intravenously in isotonic solution. We used radioactive iron Fe^{59} (half life 45 days) as $FeCl_3$ (specific activity 1 μc./mg. Fe) and glycine -2-C^{14} in isotonic solution. Goat 1 received 4.9 μc. Fe^{59}/Kg. bodyweight (1 day old, 3250 Gm.); goat 2, 2.4 μc./Kg. (2 days old, 4150 gram); goat 3, 24.4 μc. C^{14}/Kg. (5 days old, 3700 Gm.).

Two other newborn goats were treated with exchange transfusion with the blood of the mother. This technic was the same as in children suffering from erythroblastosis fetalis, except that the animals were anesthetized with nembutal and the exchange was carried out via the vena jugularis externa. These animals were fed in the same way as the untreated goats. The average weight gain was much lower; during the first month they did not grow at all, after that the average gain in bodyweight was about 60 Gm./day. Radioactive iron was injected intravenously one day after the exchange transfusion. Goat 4 received 4.2 μc. Fe^{59}/Kg. bodyweight (5 days old, 5100 Gm.); goat 5, 4.35 μc. Fe^{59}/Kg. (5 days old, 4550 Gm.).

Estimation of the percentage of Hb-F and the isolation of the proteins:

Samples of heparinized blood were taken from the vena jugularis externa. After removing the plasma and washing the erythrocytes, hemolysis was carried out in the usual way by adding distilled water (one vol.) and toluene (0.4 vol.). The hemoglobin solutions obtained were purified as described for human hemoglobin.[19] For the determination of percentage of fetal hemoglobin in a freshly prepared hemoglobin sample the same spectrophotometric alkali denaturation method was used as described by Jonxis and Visser[7] for human hemo-

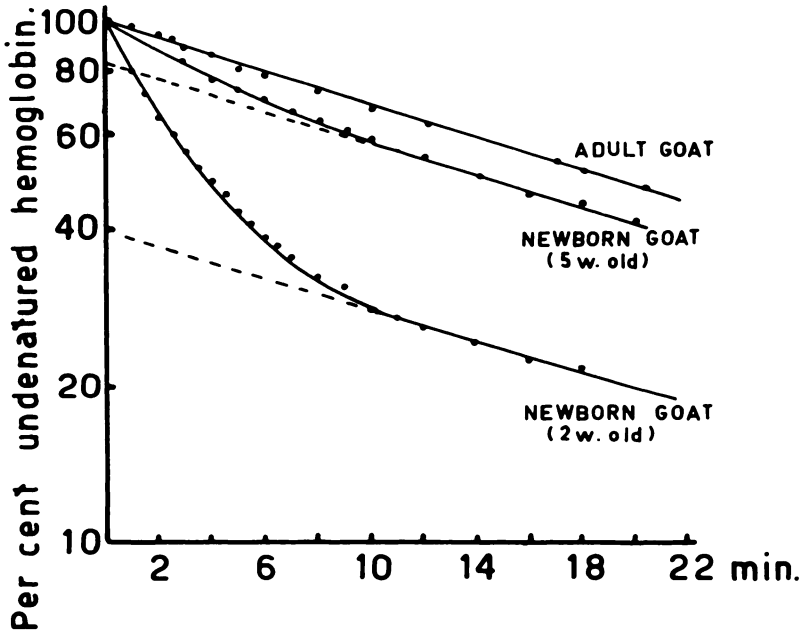

Fig. 1.—Alkali denaturation curves of the hemoglobin of adult and newborn goats.

globin, except that pure hemoglobin solutions were denatured with 0.1 N NaOH. It is remarkable that the adult hemoglobin of the goat is more resistant than the fetal component.[3, 20] As shown in figure 1, the fetal hemoglobin is denaturated completely after 12 minutes, whereafter the amount of normal adult Hb can be calculated in the usual way by extrapolation to zero time.

The same denaturation method was used for the isolation of the different fractions. Dialyzed hemoglobin solutions were denaturated with 0.1 N NaOH during 12 minutes. The denaturated protein was precipitated with ammonium sulfate after neutralization with hydrochloric acid. This precipitate was isolated and dialyzed against distilled water during 48 hours. This fraction consisted of fetal hemoglobin and a small amount of adult hemoglobin, also denaturated during 12 minutes. The percentual amount of Hb-A in this fraction was calculated from the alkali denaturation curves. The hemoglobin remaining in solution and consisting of only Hb-A was isolated by precipitation at a pH of 3 and finally dialyzed against distilled water during 48 hours.

Determination of specific activity:

Both isolated proteins were counted for Fe^{59} specific activity after drying on aluminium disks, with an Amperex end-window counter, type 200 cB (background 10 c.p.m.). Usually counts of 10 to 20 times background and higher were recorded, counts under three times background were not made. All estimations were made in duplicate. In the case of radioactive C^{14} the protein samples were counted also on flat aluminum disks, but with a Siemens end-window counter (mica window thickness 0.7 mg./cm.^{2}). The background of this counter was 11 c.p.m. Sample counts were 40 to 50 times background. All estimations were made in duplicate, with the usual corrections for self-absorption.

Results and Discussion

In figure 2 are shown at first the curves of the disappearance of the fetal hemoglobin in the blood of newborn goats after birth. About 60 per cent Hb-F is present on the first day. After 60 days only 5 per cent of this component remained. During the first few days after exchange transfusion the Hb-F was not detected in the circulating blood, but in the next few weeks up to 20 per cent of this component was found.

In the same figure the curves are given of the uptake of radioiron and of radioactive glycine in the adult and also in the fetal hemoglobin for the normal newborn goats (1, 2, 3) and for the two goats treated with exchange transfusions (4, 5). Maxima of specific activity of radioiron in adult hemoglobin occurred in untreated newborn goats at the 12th day (1, 2), of C^{14} at the 6th day (3), and of radioiron in "exchange goats" at the 8th day. These maxima are in good agreement with the data obtained by Helwig and Greenberg[21] who have found a maximum incorporation of radioiron in the hemoglobin of the adult guinea pig after about 20 days and of C^{14} (as glycine-2-C^{14}) after about 6 days. Maxima of specific activity of radioiron and C^{14} in the fetal hemoglobin in the untreated goats occurred at a later time as compared with the adult hemoglobin, namely after 32 and 24 days respectively, while in the "exchange goats" the maximum for Fe^{59} in the Hb-F is attained at about the same time as for the Hb-A (8 days). The maxima of activity of the fetal hemoglobin in "exchange goats" exceed more than 5 times those in the untreated goats 1 and 2. The large difference in time between the maxima of specific activity for radioiron and radioactive C^{14} both for adult and fetal hemoglobin will be studied later on; the results of these investigations will be given in the near future.

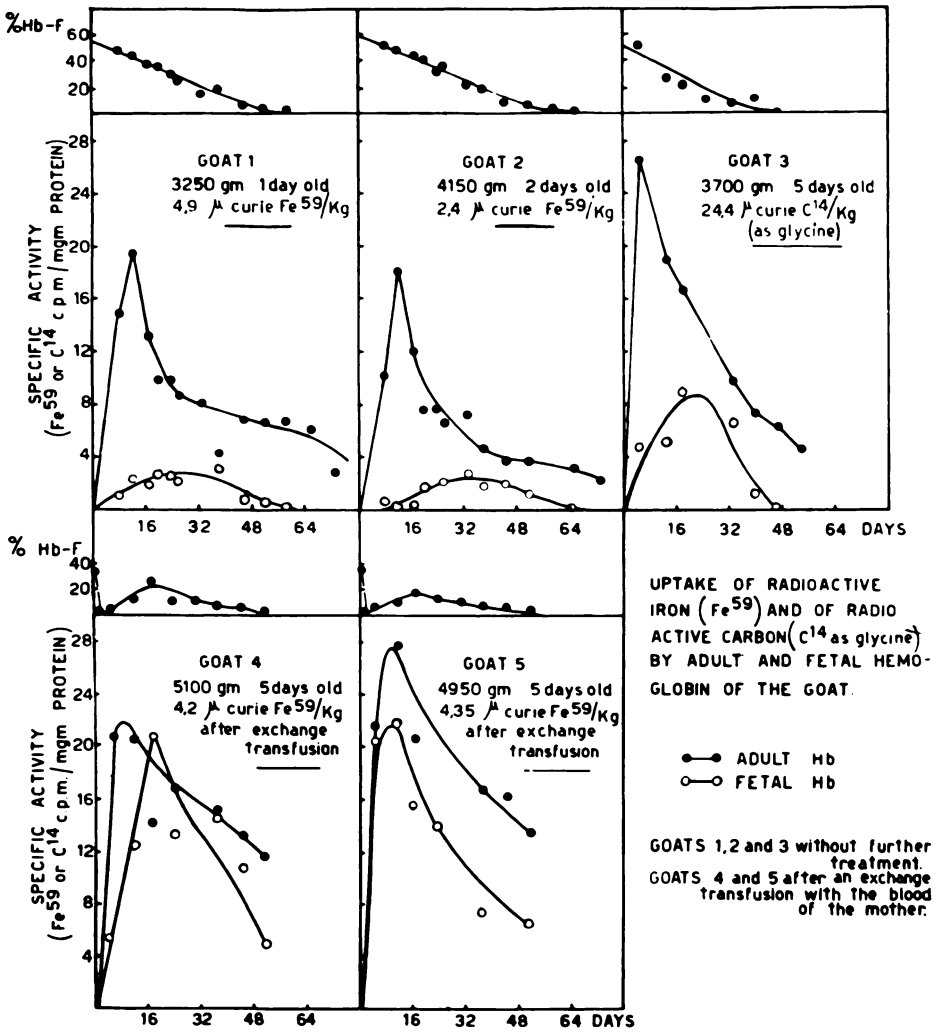

FIGURE 2

The reappearance of Hb-F in the goats after exchange transfusion and the incorporation of radioactive Fe^{59} and C^{14} in this fraction and in the fetal hemoglobin of the untreated goats are direct indications that indeed fetal hemoglobin is synthesized after birth. The much higher specific activity of this fetal hemoglobin in the "exchange goats" and the fact that the maximum incorporation of iron in this fraction is reached already after 8 days instead of 32 days may be explained by a highly activated synthesis of the Hb-F caused by the increased destruction of erythrocytes and of hemoglobin after the exchange transfusion.

It is also possible to make some calculations about the total body-specific activity for fetal and adult hemoglobin. These are to be obtained by multiplying the specific activity per milligram of hemoglobin with the total amount of hemoglobin present. As the animals gain in body weight during the experiment it is

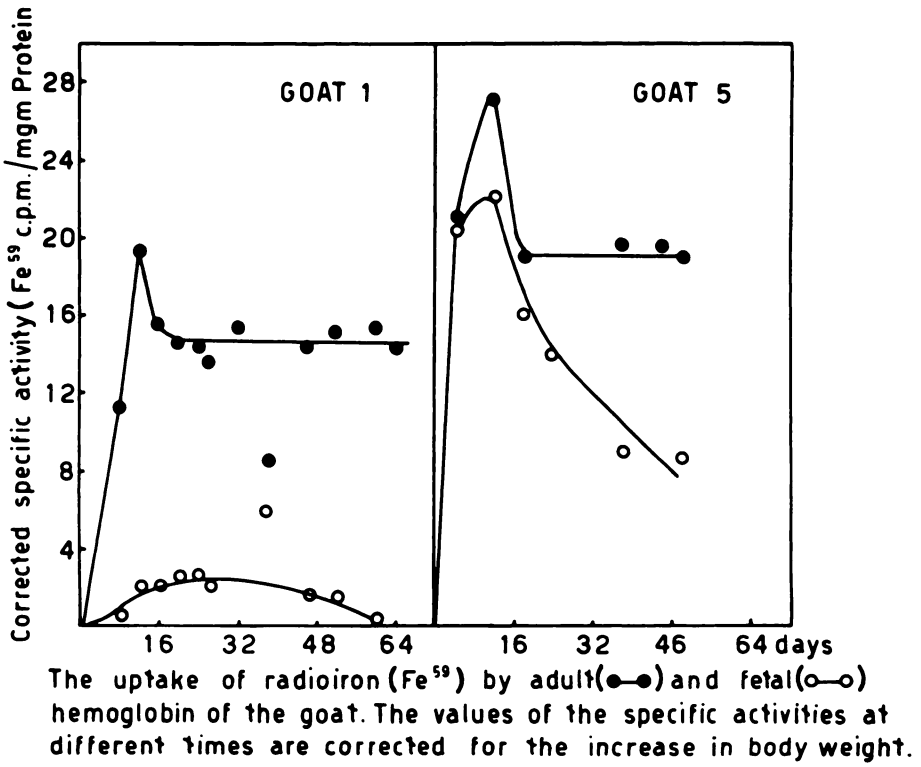

FIGURE 3

necessary to correct this value with the gain in blood volume. Assuming an equivalent increase of blood volume and of body weight, we have based these corrections on the increase in body weight. The results of these calculations for the adult and fetal hemoglobins in goats 1 and 5 are given in figure 3. The horizontal levels of adult hemoglobin after the maxima of specific activity point out a re-utilization of the radioactive iron. The declining curves of the total specific activity of fetal hemoglobin after the maxima can be explained by the decreasing synthesis of Hb-F after birth.

SUMMARY

Postnatal synthesis of fetal and adult hemoglobin was studied with radioactive iron (Fe^{59}) and glycine-2-C^{14} in newborn goats.

At birth about 60 per cent of Hb-F is present in newborn goats; after 60 days only about 5 per cent is found. Two goats were treated with "exchange transfusions" with blood of the mother. Using an alkali denaturation method the fetal hemoglobin was not demonstrable during the first few days after this treatment, but 20 days later about 20 per cent was present in circulating blood.

Maxima of radioiron-specific activity in adult hemoglobin occurred in untreated newborn goats at the 12th day, of C^{14} at the sixth day; in "exchange goats" for radioiron at the 8th day. The fetal hemoglobin maxima of specific ac-

tivity occurred in the untreated goats resp. after 32 and 24 days, in "exchange goats" after 8 days.

The reappearance of Hb-F in "exchange goats" and the specific activity of Hb-F are direct indications for a synthesis of this component after birth in this species.

Summario in Interlingua

Le synthese postnatal de hemoglobina fetal e adulte esseva studiate per medio de radioactive ferro Fe^{59} e glycina-2-C^{14} in capras neonate.

Al nascentia capras ha circa 60 pro cento de hemoglobina fetal. Post 60 dies, hemoglobina fetal amonta a solmente 5 pro cento. Duo capras esseva tractate con "transfusiones de excambio" con sanguine ab lor matres. Le methodo a disnaturation per alcali non demonstrava ulle presentia de hemoglobina fetal durante plure dies post iste tractamento, sed 20 dies plus tarde, le hemoglobina fetal amontava a circa 20 pro cento in sanguine circulante.

Maximos de activitate specific de ferro radioactive in hemoglobina adulte occurreva in non-tractate capras neonate le dece-secunde die e de carbon radioactive le sexte die. In capras "a excambio" el maximo de ferro radioactive occurreva le octave die. In hemoglobina fetal, le correspondente maximos occurreva post 32, 24, e 8 dies, respectivemente.

Le re-apparition de hemoglobina fetal in "capras a excambio" e le activitate specific de hemoglobina fetal es indicationes de un synthese postnatal de iste componente in le specie investigate.

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