The Production of Hemoglobin C in Sheep Carrying the Gene for Hemoglobin A: Hematologic Aspects

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During severe blood loss anemia in sheep heterozygous for the two adult hemoglobin types, Hb A and Hb B, Hb A is replaced by a third hemoglobin type. This new hemoglobin, designated Hb C, is distinguishable from hemoglobins A and B by the lower electrophoretic mobility at alkaline pH and by characteristic mobilities in cation and anion exchange chromatography. On study of the gross structure of Hb C it was found to have two normal α polypeptide chains, but the two β polypeptide chains were altered. Analyses of the amino acid composition of the isolated chains revealed many differences between the β chains of hemoglobins A and B and those of C, while the amino acid composition of the α chains of the three hemoglobin types were very similar, if not identical. It therefore seemed reasonable to conclude that during severe blood loss anemia, production of the new β chain gradually replaces that of the β chain of Hb A, and when these new β chains are combined with normal α chains the new hemoglobin type (Hb C) results.

Although sheep heterozygous for Hb A and Hb B formed Hb C after severe blood loss anemia, no such change under similar conditions was yet observed in sheep homozygous for Hb B. This paper is a report of a study of Hb C production and changes in hematology found in two sheep homozygous for Hb A during severe blood loss anemia. Since a humoral substance may be involved in the production of the altered hemoglobin type, attempts were made with homozygous and heterozygous sheep to stimulate Hb C production by administration of cobalt, by injection of 1-thyroxine, and by injection of a protein with high erythropoietic stimulating activity isolated from human urine.

Materials and Methods

Four sheep homozygous for Hb A (numbered 90, 132, 145, and 490) of the Rambouillet breed, and three sheep heterozygous for hemoglobins A and B (numbered 10, 11, and 13)
of the Texel breed were studied. Sheep 90 and 132 were made anemic by phlebotomy. Sheep 490 was treated with the urinary protein preparation, while sheep 145 served as control for this experiment. Sheep 10 was treated with 1-thyroxin and sheep 11 and 13 with cobalt. Blood samples of additional sheep, homozygous or heterozygous for these hemoglobin types, and of newborn lambs were investigated when control data were required. Blood samples were collected in tubes containing either heparin or sequestrene. The samples collected from the sheep numbered 90, 132, 145 and 490, which were kept in Clemson, South Carolina, were mailed to Augusta, Georgia, without additional treatment. Since sheep 10, 11 and 13 were kept in The Netherlands, red blood cells for hemoglobin studies were washed and suspended in 0.9 per cent NaCl solution containing streptomycin and penicillin and sent to the United States by air mail. Hematologic data on these samples were obtained through the courtesy of Dr. K. Punt and Miss J. Bos of the Division of Hematology, State University of Utrecht, The Netherlands.

Standard laboratory procedures were used for routine hematologic data, which included total hemoglobin concentration, packed cell volume (PCV), red and white blood cell counts and reticulocytes. Red blood cell diameter distribution curves (Price-Jones curves) were determined following the procedure given by Wintrobe; the number of cells measured varied from 300 to 500.

A procedure was also developed to demonstrate the various hemoglobin components inside the red cell which was a slight modification of the "acid elution" technic for the demonstration of human fetal hemoglobin as developed by Betke and Kleihauer.

A thin blood smear was prepared preferably from a freshly collected blood sample (not older than 24 hours) and immediately fixed in an ethanol solution for 5 minutes. The alcohol concentration was found to be quite critical; Hb A (or Hb C) and Hb B were best differentiated with an 80 volumes per cent ethanol solution, while demonstration of the fetal hemoglobin type and of "acid resistant" red cells in blood samples from severely anemic animals required 84 volumes per cent ethanol.

The slides were then washed in distilled water for 30 seconds. The hemoglobin was eluted at 37°C using an 0.1 M citric acid-0.2 M sodium phosphate buffer, pH 3.3, with constant stirring for 6 minutes. Several variations in pH (from 2.7 to 3.7) or in elution times (from 2 to 10 minutes) were tested, but proved to be less satisfactory.

The slides were again washed in distilled water for 30 seconds. The cells were stained for 3 minutes in Ehrlich's acid hematoxylin and after washing with distilled water for 3 minutes in an 0.1 per cent solution of erythrosin in water.

Photographs of slides were made with a Zeiss photo microscope; exposure setting 5; film panatomic X Kodak #58 green filter.

The type of hemoglobin was studied by starch gel electrophoresis following the previously outlined procedure. DEAE-Cellulose chromatography was the technic selected for the quantitation of the various hemoglobin components; details of this procedure have been described in previous communications. Red cell hemolysates obtained from sheep homozygous for Hb A, which also contained variable amounts of Hb C, were analyzed similarly except that shorter columns (15 × 0.9 cm.) were used. The Hb C was eluted with an 0.005 M sodium phosphate buffer, pH 8.6, while the Hb A was recovered with the use of an 0.01 M sodium phosphate buffer, pH 6.5, to which 0.3 M NaCl was added.

The oxygen affinities and the Bohr effects of various blood samples and hemolysates were determined following the procedures used in studies of human hemoglobins.

**Results**

**Production of Hb C in Sheep Homozygous for Hb A during Severe Blood Loss Anemia**

The two adult sheep, 90 and 132, were bled twice a week, 250 ml. for 14 weeks and 500 ml. for 16 weeks. During the following 9 weeks the twice-weekly 500 ml. blood withdrawal was continued, but for sheep 90 the with-
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Fig. 1.—The production of Hb C in homozygous hemoglobin A sheep during severe blood loss anemia. ○ sheep 90; ● sheep 132.

drawal was increased to 750 ml. Once each week a small blood sample was taken before the major bleeding in order to determine the various red blood cell parameters and the types and amounts of hemoglobin components present. The results are in part presented in Figure 1; the variations observed in the relations between red cell counts, hemoglobin concentrations and packed cell volumes will be presented later. During the first bleeding period, the total hemoglobin level in sheep 90 dropped from 11.8 Gm. per cent to a level of 8.5, which remained constant during the experiment; the value for sheep 132 underwent a similar drop from 13.3 Gm. per cent to a value fluctuating at about 10 Gm. per cent. A large quantity of Hb C was produced in sheep 90 (highest level 61 per cent after 8 weeks), while only small quantities (maximum value 6 per cent) were found in the blood of sheep 132. A surprising decrease in the level of Hb C in sheep 90 and to a lesser extent in sheep 132 was observed during the later part of the first period, despite the fact that the hemoglobin level was maintained at a constant low level (sheep 90) or even further decreased (sheep 132). The hematologic data at the start of the second period of bleeding (samples collected after 13 weeks) were similar in sheep 90 and 132: total hemoglobin 9.0 and 9.0 Gm. per cent, PCV 29 and 28 per cent, RBC 7.35 and 7.50 × 10⁶ per mm.³, reticulocytes 2.0 and 0.7 per cent; WBC
Fig. 2.—Starch gel electrophoretic patterns at pH 8.1 of the hemoglobin of sheep 90 during severe blood loss anemia. Samples numbered 1, 4, 5, 6, 7, and 11 were collected 1, 8, 10, 31, 36 and 38 weeks after the start of the experiment. Samples 2 sheep Hb B; Sample 3 human Hb A; sample 8 homozygous Hb B lamb; sample 9 homozygous Hb A lamb; sample 10 artificial mixture of sample 11 and the hemoglobin of a control Hb A sheep.

4480 and 5700 per mm. The increase in volume of blood removed during the second period resulted in a further decrease of the total hemoglobin levels; both sheep maintained levels between 7 and 8 Gm. per cent during the remainder of this period. Again a transient increase in the production of Hb C was observed in sheep 90; however, the maximum level reached (34 per cent after 19 weeks) was considerably less than that found in the first period. Sheep 132 responded by a slow rise in Hb C level which was maintained at approximately 30 per cent during the additional 9 weeks of bleeding. It was found necessary to increase the bleeding from sheep 90 to 1500 ml. each week for an additional period to replace Hb A entirely by Hb C. No Hb A was demonstrable in the blood of this animal 7 weeks after the start of this severe treatment and it was absent during the remaining 3 weeks of this bleeding period. The total hemoglobin level varied during this period from 6.2 to 5.1 Gm. per cent with PCV values of 14 to 20 per cent, RBC values of 3.7 to $4.8 \times 10^6$ per mm.$^3$, while the percentages of reticulocytes never exceeded 10 per cent. Both animals recovered rapidly from their anemia at the end of the experiment; there was a continued synthesis of Hb C, although a steady decrease in its level was observed. Figure 2 presents starch gel electrophoretic patterns of the hemoglobin components as they were found in blood samples of sheep 90 collected at various times during this experiment.
Fig. 3.—The demonstration of hemoglobin within the erythrocytes after exposure to an acid buffer solution. a. homozygous Hb A sheep; b. homozygous Hb B sheep; c. mixture of a and b; d. heterozygous Hb A-Hb B sheep. The blood smears were fixed with 80 per cent alcohol.

Hematologic Changes Observed During Severe Blood Loss Anemia

Figures 3 and 4 are photographs of red cell preparations treated with the acid elution technic to demonstrate the various hemoglobin components within the red blood cells. Figure 3 shows blood smears from an untreated homozygous Hb A sheep (Fig. 3a), from an untreated homozygous Hb B sheep (Fig. 3b), of an artificial mixture of the two blood samples (Fig. 3c) and from a sheep heterozygous for both hemoglobin types (Fig. 3d). All four blood smears were eluted under identical circumstances after fixation in an 80 volume per cent alcohol solution. The results indicate that Hb A is more resistant than Hb B to the acid elution, and that both hemoglobin types are likely to be present in each red blood cell of a heterozygous animal.

Figure 4a illustrates the importance of control of the concentration of the alcohol solution for the differentiation of Hb A and Hb B inside the erythrocytes. The same artificial mixture of blood samples of a homozygous Hb A sheep and of a homozygous Hb B sheep was used as that shown in Figure 3c; however, the cells were fixed with an 84 rather than an 80 volume per cent alcohol solution. With this higher alcohol concentration it was difficult to determine which red cells contained Hb A and which Hb B. Figure 4b shows a blood smear of a newborn lamb; the fetal hemoglobin was found highly
Fig. 4.—The demonstration of hemoglobin within the erythrocytes after exposure to an acid buffer solution. a. mixture of blood samples obtained from a homozygous Hb A sheep and a homozygous Hb B sheep; b. newborn lamb; c. blood sample from sheep 90 (32 weeks after start of the experiment); d. mixture of blood samples used in the experiments b and c and of a blood sample obtained from a homozygous Hb B sheep. The blood smears were fixed with 84 per cent alcohol.

resistant to the acid elution as is human fetal hemoglobin.² The demonstration of Hb F inside the cells was less satisfactory at an 80 per cent alcohol fixation. Several attempts to demonstrate Hb C in a similar way were unsuccessful; this hemoglobin type behaved like Hb A at both alcohol concentrations. An example is shown in Figure 4c which illustrates a smear made from a blood sample, collected from sheep 90, 32 weeks after the start of the experiment (Hb C: 68 per cent). The majority of red blood cells behaved as did the cells of an untreated homozygous Hb A animal. Several blood smears showed moderately acid resistant cells, which were also characterized by their increased size. The number of these cells was relatively small and never exceeded 8 per cent, which excluded the possibility that these cells were the only cells containing Hb C. Figure 4d summarizes the behavior of the red blood cells from various sources; it is a photograph of a smear prepared from a mixture of blood from sheep 90 (same sample as used in Figure 4c), of blood from a newborn lamb and of blood from a homozygous Hb B sheep. The red blood cells containing Hb B which were not resistant to treatment with acid, the slightly more resistant Hb A (or Hb C) containing red cells, and the Hb F containing red
Fig. 5.—Hematologic data of sheep 90 during the period of 27 through 36 weeks after the start of the experiment.

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found to be approximately 4.7 microns, which is in accordance with data reported in the literature.\textsuperscript{17} The Price-Jones curves of red cells from newborn lambs indicate the existence of a two-cell population; the fetal hemoglobin containing cells are likely to be slightly increased in size. The decrease in red cell diameter during severe blood loss anemia was also easily demonstrable, although the actual difference (0.1-0.3 micron) was rather small. Increased numbers of red cells with larger diameter (5.0 micron) were noted during the development of the anemia; it seems likely that these cells, which are larger than the Hb F containing cells found in the blood of newborn lambs, are the same as those observed by the acid elution technique.

Similar changes in the hematologic values were also found in a sheep homozygous for Hb B and in a sheep heterozygous for both hemoglobin types. The hematologic data observed during and after the bleeding experiments with these two animals have in part been described before.\textsuperscript{19} The following are additional observations: (1) a linear relation existed between the total hemoglobin levels and the hematocrit values; this relation was the same for the two animals in both phases of the experiment. (2) The total hemoglobin levels and the hematocrit values during the latter part of the recovery phase were significantly lower in the animal with Hb B than in the animal with Hb AB. (3) The total red blood cell counts in the two animals increased slightly with the decrease in hemoglobin level during the anemic phase. During the recovery phase of the anemia there was a large, persistently increasing production of red blood cells in both animals, which is linearly related to the total hemoglobin level. The values observed in the latter part of the recovery phase for the sheep with Hb B were also lower than those seen in the animal with Hb AB. The maximal percentages of reticulocytes were 11.5 per cent and 4.5 per cent for the sheep with hemoglobin AB and B, respectively; the
percentages of reticulocytes during the recovery phase never exceeded 1 per cent.

The physiologic properties of whole blood samples and of dialyzed red cell hemolysates were studied by determining the oxygen equilibria after equilibration with oxygen at various tensions. The pCO₂ values used in the determinations of the oxygen dissociation curves of total blood samples varied from 0 to 100 mm. Hg in different experiments. A pCO₂ of 40 mm. Hg was used when freshly prepared hemolysates were studied; these hemolysates were dialyzed against 0.1 M potassium phosphate buffers of varying pH values for 24 hours at 4 °C. Examples of results obtained are presented in Figure 7, in which the logarithms of the P₅₀ values—i.e., the values of the pO₂ at which 50 per cent saturation with oxygen was observed—are plotted against the pH values (measured at 37 °C.). The observed oxygen affinity values of blood samples and hemolysates obtained from untreated sheep homozygous for Hb B and from untreated sheep homozygous for Hb A confirmed earlier observations of an increased oxygen affinity of Hb A containing samples. The data also demonstrated that the Bohr effects of the two types of blood or their hemolysates are identical. When Hb A was almost completely replaced by Hb C (the blood sample studied was collected from sheep 90 thirty-five weeks after the start of the experiment and contained 96 per cent Hb C with a total hemoglobin of 6.2 Gm. per cent), a considerable increase in oxygen affinity of total blood was noted. This phenomenon was not observed when red blood cell hemolysates were studied. The Bohr effects of Hb C containing blood samples and hemolysates were similar to those found for the samples collected from the untreated homozygous Hb A or Hb B sheep and different from that collected from newborn lambs.
Is a Humoral Factor Involved in the Production of Hb C?

The results of the bleeding experiment with the homozygous Hb A sheep 90 (Fig. 1) demonstrated a considerable production of Hb C during the relatively short period of continuous decrease of the total hemoglobin level. The synthesis of this hemoglobin type decreased when the total hemoglobin level was maintained at a rather low, but constant, level and was only continuously produced when the total hemoglobin reached values of 6 Gm. per cent or less. These observations could indicate the existence of a humoral stimulus of the production of Hb C. In line with this hypothesis, the possible synthesis of Hb C was studied in sheep heterozygous for Hb A and Hb B during treatment with either cobaltous chloride or 1-thyroxin, both of which are known or believed to act as stimuli of erythropoiesis in rats and dogs.4,5,12,14,20 Also, a sheep homozygous for Hb A was treated with a purified protein extract with high erythropoietic activity of human urinary origin.

Two heterozygous sheep (57 and 54.2 Kg, body wt) received each day 3.3 mg. of cobalt (as cobaltous chloride) orally for 50 days. This dosage was based on the observations of Becker and Smith1 as to the cobalt tolerance studies in sheep. The two sheep withstood the daily dosage without any apparent undesirable effects; a slight weight increase was noted after 50 days (body weights 66 and 61 Kg, respectively). No significant changes in the total hemoglobin level, in PCV and RBC levels, was observed. The percentages of Hb C in the blood of both animals were insignificant; all values, with the exception of one, fell within the limit of accuracy of the determination, which is 0.5 per cent for samples containing the two hemoglobin types A and B. The per cent of Hb A and Hb B did not change significantly during the period of cobalt treatment. One sheep heterozygous for Hb A and Hb B was used for the 1-thyroxin experiment. This hormone was dissolved in a propylene glycol-alcohol-water mixture and given immediately by intramuscular injection. A dosage of 4 mg./Kg. body weight was selected in accordance with the findings of Robertson and Broome;16 this amount was injected on each of two consecutive days. The effect of the 1-thyroxin administration on the basal metabolic activity was quite dramatic; the respiration frequency increased from 30 per minute to a maximum of 145 per minute after 6 days and continued to be increased over a long period of time. The rectal temperature rose from 39.2° C to a maximum of 41° C. The body weight decreased from an initial 85 Kg. to 75 Kg. (after 9 days) to 70 Kg. (after 16 days); thereafter, a slow increase was observed. The total level of Hb, PCV and RBC showed an initial increase of approximately 10 per cent, the increase lasting for about one week. This increase was followed by a decrease; thereafter, approximately constant levels of all three parameters were maintained. No reticulocytosis was observed. The initial values of Hb C were found to be slightly higher than 0.5 per cent, the limit of accuracy of the determination. A questionable increase of the Hb C level was observed during the 1-thyroxin treatment; a level of approximately 1 per cent was maintained for a considerable length of time. The per cents of Hb A and Hb B were not affected.

One homozygous Hb A lamb, numbered 490, was used for the third experi-
ment. This animal was approximately 5 months old and weighed 34.5 Kg. No fetal hemoglobin was present at the start of the experiment. A homozygous Hb A adult ewe, numbered 145 and weighing 75.5 Kg., served as control. Both animals were kept indoors and fed the same constant diet. The human urinary concentrate used in this experiment was prepared from urine of a patient with refractory anemia of unknown etiology following a procedure outlined previously. The final product contained 1860 mg. of protein with an erythropoietic activity of 1.6 units per mg. of protein. Five doses, 372 mg. of protein each, each dissolved in 20 ml. of sterile 0.9 Gm. per cent NaCl solution were given on 5 consecutive days by injecting half of one dose intraperitoneally in the morning and the remainder in the afternoon. The control sheep 145 was treated similarly with approximate injections of 0.9 Gm. per cent NaCl solution. The lamb 490 tolerated the injections reasonably well; its weight decreased to 30.5 Kg. (after 7 days) but increased steadily during the remainder of the experiment. A slight, transient rise in rectal temperature was noted. On the 15th and 16th day after the start of the experiment, lamb 490 was again treated with a similar extract. This second concentrate was prepared from 30 liters of urine collected from sheep 90 during the period of severe anemia (Fig. 1). The method used was that described by Lewis, Alford and Lange. The final concentrate contained an erythropoietic activity of 0.12 units per mg. of protein; two doses, each containing 4.5 Gm. of protein, were administered in the same manner as the human urinary extract. Small blood samples from both sheep were collected at frequent intervals and studied for total hemoglobin, PCV, RBC, WBC, reticulocytes and the percentage of Hb C. The results of these studies are presented in Fig. 8. A slight increase in the total hemoglobin and PCV values was observed; no reticulocytosis was produced. The amount of Hb C showed a slight, but definite, increase which was maximal 8 days after the last injection of the human urinary concentrate. The maximal increase observed was small, namely 3 to 4 times the value of 0.3 per cent, which is considered the limit of accuracy of the chromatographic procedure used for samples containing only Hb A. The Hb C values observed in the control sheep 145 remained within the limit of 0.3 per cent. The second injection with (sheep) urinary concentrate also resulted in a questionable increase in the total hemoglobin and PCV values, while no effect on the per cent of Hb C was noted.

**DISCUSSION**

It is apparent from the results that the Hb A of a homozygous sheep can be replaced entirely by a different hemoglobin type, Hb C, during severe blood loss anemia; the data presented confirm the results of similar observations made earlier in heterozygous sheep. Sheep 90 is very likely the first mammal in which the type of hemoglobin was changed completely for another due to an experimental influence on the mechanism involved in hemoglobin synthesis. The manner in which the severe blood loss stimulated this change in hemoglobin type is a matter for considerable speculation. At first sight it might be considered possible to attribute the change directly to a decreased supply of
Fig. 8.—The effect of urinary protein extracts with erythropoietic activity on the hemoglobin C production in a homozygous Hb A sheep. ○ sheep 490. ● control sheep 145.

oxygen to the tissues. This seems doubtful, however, since the production of Hb C decreases steadily when the total hemoglobin level is maintained at constant, but low, levels. It is tempting from the responses of the two homozygous Hb A sheep to severe bleeding to consider the possibility of the production of humoral factor(s) inducing the formation of Hb C. In assuming such a possibility it would seem likely that the extent of the Hb C formation is directly related to the amount of such factor(s) produced, which in turn depends on the severity of the anemia. The suggestion that such possible factor(s) is (are) directly related to or even identical with factors causing an augmentation of erythropoiesis is extremely speculative. The various additional experiments presented above have demonstrated that minor increases in Hb C production will occur as a result of the administration of a human urinary concentration with relatively high erythropoietic activity. It seems of importance to extend such studies to a larger group of animals and to use additional stimuli; the size of the animal, the duration and technical complications of such experiments are, however, limiting factors of considerable magnitude.

Although there are some indications of the occurrence of factor(s) inducing the synthesis of Hb C, this does not explain the switch-over mechanism responsible for the described phenomenon. Detailed structural analysis of the various hemoglobin types will be required to determine exactly the change in
the hemoglobin produced; results of such studies will be presented in due course.

It has been reported recently that there is a correlation between the type of hemoglobin and the maximal PCV values in adult sheep, suggesting that these two phenomena are both under genetic control and significantly associated. Earlier observations have indicated that similar differences in the total hemoglobin levels and in the numbers of red blood cells do exist in sheep with different hemoglobin types. The results of hematologic studies in anemic sheep do support these earlier observations indicating that all three parameters of the hemoglobin synthesizing system rather than the PCV values alone are significantly associated with the type of hemoglobin produced. The differences in these values as they are observed in sheep with different hemoglobins are likely to be secondary to this genetically controlled character. The stimulus to an increased production of red blood cells of comparable size and hemoglobin concentration in sheep with Hb A could be the decreased ability of Hb A to release oxygen to the tissues due to its increased affinity for oxygen.

The production of large quantities of red blood cells of small size without a notable production of cells identifiable as reticulocytes does suggest that the crisis provoked in sheep by the bleeding process stimulates red cell production primarily by the normal pathway. A second pathway recently suggested by Borsook, Lingrel, Scaro and Mellett—namely, the formation of reticulocytes derived directly from polychromatic cells—could explain the occurrence of (relatively small) quantities of red blood cells of enlarged size in the anemic homozygous Hb A sheep. An interesting property of these enlarged cells is the increased resistance of their hemoglobin to elution with an acid buffer solution. Their numbers were found to correspond reasonably well with the numbers of reticulocytes; experiments to demonstrate basophilic material in these cells after exposure to the acid buffer were, however, unsuccessful.

Application of the acid elution technics to blood samples of sheep heterozygous for both hemoglobin types showed that all red blood cells contained the two hemoglobin types. This equal distribution is comparable with that recently demonstrated for Hb A and Hb S in red blood cells of patients with sickle cell trait. This observation lends support to the hypothesis obtained from pedigrees and survey data that the occurrence of the two hemoglobin types are genetically determined by a pair of alleles at a single locus. (For references regarding pedigree data, see reference 19.) Red blood cells containing Hb C were found to behave as Hb A containing erythrocytes, thus preventing their demonstration in blood samples of anemic homozygous Hb A sheep by the acid elution technic. Fetal red blood cells were easily demonstrable; the resistance of sheep fetal hemoglobin to the elution with acid buffer was found to be comparable to that of human fetal hemoglobin. It is not possible to exclude with certainty the possibility that the acid resistance of the hemoglobin found in the enlarged red cells in blood samples of the severely anemic homozygous Hb A sheep is the result of the presence of notable quantities of fetal hemoglobin; however, the absence of demonstrable amounts of fetal
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hemoglobin in hemolysates prepared from these blood samples certainly does not support such a possibility.

Summary

The first part of this study describes the hematologic and physiologic changes observed in sheep homozygous for the hemoglobin A during severe blood loss anemia. It was found possible thus experimentally to replace Hb A entirely with a new hemoglobin type, Hb C. The following additional observations were made: (1) Hb C could not be distinguished from Hb A by submitting the appropriate red blood cells to an “acid elution” technic. These two hemoglobin types were found to be more resistant to this treatment than a second adult hemoglobin type, Hb B, while the fetal hemoglobin of the newborn lamb was found to be highly resistant. In sheep heterozygous for Hb A and Hb B, both hemoglobin types were equally distributed among all red blood cells. (2) During stages of severe blood loss relatively small quantities of acid resistant red blood cells of larger size were demonstrable in homozygous Hb A sheep; these cells were considered to be reticulocytes. Additional observations regarding variations in red cell parameters are also presented. (3) The oxygen affinities and the Bohr effects of blood samples and red cell hemolysates containing over 90 per cent Hb C are presented and compared with those of samples containing only Hb A, Hb B or the hemoglobin of the newborn lamb.

Attempts to produce Hb C in sheep homozygous for Hb A by means other than phlebotomy are described in the second part of this report. Small amounts of Hb C were demonstrable in a sheep homozygous for Hb A after repeated injections of a urinary extract of human origin with high erythropoietic activity. Administration of cobalt and of thyroxin did not result in the formation of significant amounts of Hb C.

Summario in Interlingua

Le prime parte del presente studio describe le alterationes hematologic e physiologic observate in oves homozygotic pro hemoglobina A durante anemia sever per perdita de sanguine. Esseva trovate possibile reimplaciar experimentalmente per iste methodo hemoglobina A completely per un novo typo de hemoglobina, hemoglobina C. Esseva facite le sequente observationes additional: (1) Hemoglobina C non poteva esseva distinguite ab hemoglobina A per submitter le appropriate erythrocytos a un technica de “elution acide.” Esseva trovate que iste duo typos de hemoglobina esseva plus resistente contra iste tractamento que un secunde typo de hemoglobina adulte, hemoglobina B, durante que le hemoglobina fetal de agnos neonate se provava altemente resistente. In oves heterozygotic pro hemoglobina A e hemoglobina B, ambe typos de hemoglobina esseva distribuite equalmente inter omne le erythrocytos. (2) Durante le phases de un sever perdita de sanguine, relativemente micre quantitates del erythrocytos acido-resistentente de dimensiones major esseva demonstrabile in oves homozygotic pro hemo-
globina A. Iste cellulas esseva considerate como reticulocytos. Observationes additional relative a variationes in parametros erythrocytic es etiam presentate. (3) Le affinitates pro oxygeno e le effectos de Bohr de specimens de sanguine e de hemolysatos erythrocytic continente plus que 90 pro cento de hemoglobina C es presentate e compare con illos de specimens continente exclusivemente hemoglobina A, hemoglobina B, o le hemoglobina del agno neonate.

Es describite, in le secunde parte del presente reporto, essayos de producer hemoglobina C in oves homozygotic pro hemoglobina A per medios altere que phlebotomia. Micre quantitates de hemoglobina C esseva demonstrabile in un ove homozygotic pro hemoglobina A post repetite injectiones de un extracto urinari de origine human con alte activitate erythropoietic. Le administration de cobalt e de thyroxina non resultava in le formation de quantitates significatives de hemoglobina C.

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