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A COMPARATIVE STUDY OF THE ALKALI RESERVE OF NORMAL, PREGNANT, AND FETAL SHEEP*

In a study of the carbon dioxide exchange between the fetal and maternal bloods of goats, Keys[3] observed that the alkali reserve of the fetal blood sampled after caesarian section was higher than that of the maternal plasma obtained after the delivery of the fetus. The alkali reserve of the fetal kids fell within the range characteristic of normal barren goats, but that of the mother was in every case below the normal range. These observations, made on goats in the last 30 days of the gestation period, led him to infer that the pregnant goat is in a state of metabolic acidosis, an inference that gained support from studies by Barcroft and his collaborators[1] indicating that the position of the oxygen dissociation curve, prepared at constant CO_2 pressure with the blood of gravid goats, was to the right of the field characteristic for the curves similarly prepared with blood from individuals not gravid.

My own observations on the carbon dioxide content of fetal and maternal plasmas of sheep,[2] drawn under similar circumstances after the lambs had been delivered by caesarian section, indicated that here, too, the concentration was higher in the fetal plasma. This observation, viewed in the light of the results of Keys[3] and Barcroft *et al.*,[2] pointed to the possibility that the gravid ewe like the goat was in a state of metabolic acidosis. Hence, I determined to examine this possibility in the hope of learning something about the genesis and development of pregnancy acidosis. The results obtained form the substance of this report which deals with the alkali reserve in normal barren ewes, in gravid individuals at several stages in gestation, and fetuses in a number of different stages of development.

As the first step in the investigation the alkali reserve was determined in a series of normal ewes that were not pregnant and had not recently been so, in an effort to establish the level and the range of variation characteristic of individuals kept under usual farm conditions. Those objectives obtained, I next followed the changes in the alkali reserve in a small series of ewes, with appropriate controls, before, during, and after the gestation period.

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Inasmuch as the results of the second step in the investigation indicated that the alkali reserve of the blood of the pregnant ewes, as sampled, did not fall outside the range of variation of the controls, I was obliged to consider the possibility that the lower carbon dioxide content of the maternal blood at delivery by caesarian section might be due—in my earlier cases and in those of Keys[8] and Barcroft *et al.*[2] as well—to some circumstance associated with the preparation for and/or the delivery of the fetus by caesarian section.

The testing of this possibility formed the third stage in this program of study. Comparisons were made between the alkali reserves of plasmas obtained from blood samples drawn before and after preparation for the laparotomy with spinal or nembutal anesthesia. The results indicated quite clearly that the alkali reserve of the ewe was lowered by the procedures antecedent to the laparotomy—the administration of the anesthetic and placement on the back on the operating table. Clearly the final stage, the comparison of the alkali reserve of the fetus and the mother, could not be considered representative of circumstances normally occurring *in utero,* except in those cases in which the reserve of the mother was not substantially altered by the procedures associated with the delivery of the fetus. When the ewes for this part of the study were chosen for their quiet temperament and the fetus was delivered after the application of a local anesthetic to the abdominal wall, with the ewe lying on her side, her head gently supported by an attendent, I succeeded in obtaining samples for comparison in which the maternal alkali reserve was not altered more than three volumes per cent from the time the first sample was drawn in the pen until the end of the experiment. The data so obtained I have taken to be representative of normal circumstances *in utero.*

MATERIALS AND METHODS

For these studies, as in my earlier ones, I have used good grade or registered Dorset and Shropshire ewes; they were kept by Dr. and Mrs. Morton Loeb on their Bethany farm with their own flock. The Loebs provided a record of the breeding dates indispensable for the investigation. The ewes had the run of a good pasture with a daily ration of grain and alfalfa hay *ad libitum* during the winter months. An open barn provided shelter in foul weather.

The blood samples in these studies were drawn into an oiled syringe containing a solution of potassium oxalate and ammonium fluoride (2% $K_2C_2O_4$ and 0.6% NH_4F) in an amount such that the final mixture contained nine parts of blood and one of the anticoagulant; the results were corrected for this dilution. The alkali reserve of the true plasma was determined in each case from the carbon dioxide dissociation curves prepared in the usual manner by equilibrating oxygenated blood samples in tonometers filled with air to which varying amounts of CO_2 were added. The number of points

determined depended upon the size of the blood sample available, but was never more than five nor less than two. If only two points were to be determined the CO_2 pressures in the tonometers were adjusted to about 35 and 50 mm. Hg. respectively. Equilibration was carried out in a water bath at 39° C.; after ten minutes the gas pressure was brought to atmospheric by opening the stopcock of the tonometer with its tip just below the surface of the water in the bath and allowing the excess to escape. At the end of the equilibration period—20 minutes—the blood was removed into a syringe, lubricated with mineral oil, by driving the needle through the rubber stopper that closed the other end of the tonometer. The larger fraction of the sample was transferred to a centrifuge tube filled with oil and closed by a one-hole stopper; as the blood entered the tube, the oil escaped through the hole which was closed with a glass plug when only a thin layer of the oil remained to cover the blood. Cells and plasma were separated by centrifugation.

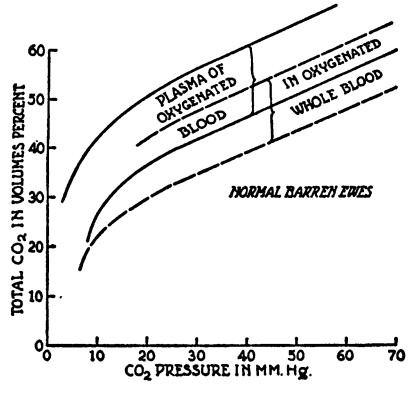

FIG. 1. A graph indicating the range of variation in the carbon dioxide dissociation curves — oxygenated whole blood and true plasma — in a series of ewes not pregnant or recently so.

The carbon dioxide content of the whole blood was determined by Van Slykes' manometric method on 0.5 cc. samples transferred directly from the syringe to the pipette. Determinations were similarly made on the plasma drawn from the centrifuge tube and transferred to the pipette by means of an oiled syringe. The concentration of CO_2 in the tonometers was determined with the Henderson-Haldane apparatus.

RESULTS

The alkali reserve in normal ewes. For the initial observations on the alkali reserve of normal ewes twelve mature individuals were available. The blood samples were drawn from the external jugular vein whilst the ewes were standing in a small pen to which they had been confined an hour or two earlier. The results obtained on the blood samples so drawn were used to construct Figure 1; the solid lines represent the field within which the points obtained from the analysis of the true plasma fell. The points obtained with whole blood fell within the area limited by the dotted lines. Taken together these data indicate that the alkali reserves of this series of ewes ranged between 46.5 and 61.0 volumes per cent—a range that would appear to be fairly representative of the normal variation to be found in ewes kept under the usual farm conditions.

The alkali reserve in pregnant ewes. Ten ewes were used in this study in which the alkali reserve was followed in each individual at approximately

tri-weekly intervals over a period of several months during which eight of them were bred and gave birth to healthy lambs; the two that were not bred served as controls. Two of the ewes bore twins, the remaining six, singlets.

The blood samples for the preparation of the CO_2 dissociation curves were drawn from the jugular vein with the ewe standing quietly in the small pen to which she had been confined for an hour or so. With but two exceptions the alkali reserve in these ewes never varied, in the period they were under study, beyond the limits described above as characteristic of the normal, non-gravid animals. The data obtained on these two exceptions —one bore twins, the other a singlet—are illustrated graphically in Figure 2, together with the data on one of the controls; they indicate that the alkali reserve of these two ewes never fell below the range found in normal ewes, but did rise above that range shortly after insemination. The significance of this rise, if it has any, is not clear, nor is that of the fall that follows in the winter months, but the latter is clearly not due to the gravid state of the ewes for it occurred in the controls as well.

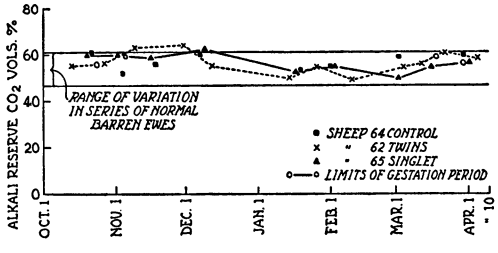

FIG. 2. A graph representing the variation in the alkali reserve of three of a series of ewes followed over months and including the gestation period.

The effects of handling and anesthetics on the alkali reserve. As these observations on the alkali reserve of pregnant ewes lent no support to the view that they were acidotic when the blood samples were drawn whilst they were standing quietly, the possibility that the low bicarbonate levels of the plasmas of goats and sheep when sampled at caesarian section was due to circumstances associated with the preparation for the operation appeared worthy of investigation. The experiments were carried out as follows: A first sample of blood was drawn from the external jugular vein while the ewe was standing quietly in her pen; the spinal anesthetic or intravenous nembutal was then given and the ewe placed on her side or back on the operating table and restrained as in preparation for the caesarian section. At selected intervals thereafter blood samples were drawn from the jugular for the preparation of carbon dioxide dissociation curves of oxygenated blood and true plasma as mentioned above. Six ewes were studied in this manner. In each case the alkali reserve of the plasma as sampled fifteen to twenty minutes after the ewe was anesthetized and restrained was between

2.5 and 10.7 volumes per cent less than that of the first plasma sample; the fall in the alkali reserve is illustrated in Figure 3. These curves were prepared with the blood of sheep 12, and the second sample was drawn twenty-two minutes after the ewe had been anesthetized with nembutal and placed on her back. In another ewe given a spinal anesthetic and placed on her side the alkali reserve fell from 52.5 to 44.1 volumes per cent in the interval of an hour. The details are given in Table 1.

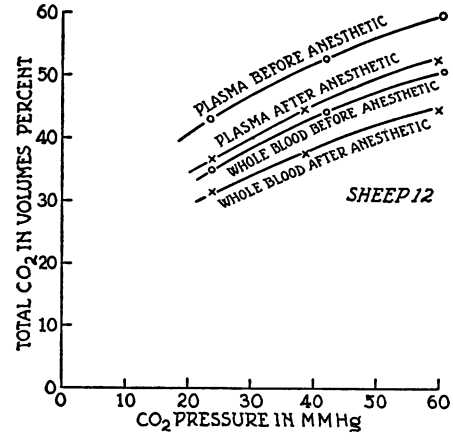

FIG. 3. The carbon dioxide dissociation curves — oxygenated blood and true plasma — prepared with samples from the jugular vein before and after administration of intravenous nembutal.

These observations demonstrate the influence of the anesthetic and handling of the ewes on their alkali reserve; further, they indicate quite clearly that inferences drawn about the relative levels of the fetal and maternal alkali reserve in pregnant sheep on data obtained from samples drawn at caesarian section may not represent the circumstances as they exist in an individual undisturbed in her pen or grazing in a pasture.

Clearly, in order to establish the relative levels of the alkali reserve of fetal and maternal plasmas in sheep as they exist at several stages of pregnancy the blood samples must be obtained under conditions in which the bicarbonate levels are not altered from normal. In the course of my efforts to obtain such samples, I assumed that circumstances that failed to alter the maternal plasma levels would be without appreciable effect on the fetal. After a number of unsuccessful trials with general anesthetics, I finally hit on a procedure by which the abdomen of the ewe could be opened, the fetus delivered, and a blood sample drawn without altering her alkali reserve more than three volumes per cent in the interval. In some cases there was no change.

The procedure was as follows: I selected a series of eight ewes that had been handled a good deal and were of a placid nature. They were quite accustomed to lying on their sides on a table with their heads cradled by an assistant and without anything to restrain their movements. The anterior abdominal wall was anesthetized with a local anesthetic, given through a new and sharp no. 20 needle. After a blood sample had been drawn from

the external jugular, the abdomen was then opened with a minimum of delay, a single incision separating all the layers and exposing the uterus. Again a single incision exposed the fetal vessels, the fetal sample was drawn, and the second sample obtained from the maternal jugular in an interval of five minutes.

TABLE 1. CHANGES IN ALKALI RESERVE FOLLOWING ADMINISTRATION OF SPINAL ANESTHETIC AND PREPARATION FOR LAPAROTOMY

Time	*Position of ewe*	*Alkali reserve vols. %*
10:45 a.m.	Standing	52.5
11:00 a.m.	Given spinal—placed on side	49.
11:20 a.m.	Lying on side	48.8
11:45 a.m.	Lying on side	44.1

TABLE 2. THE ALKALI RESERVE OF PREGNANT EWES AND THEIR FETUSES

Sheep no.	*Fetal age*	*Fetal alkali reserve*	*pH at pCO_2 40 mm. (calculated)*	*Maternal alkali reserve*	*pH at pCO_2 40 mm. (calculated)*
54	62	47.5	7.32	57.3	7.41
8	81	44.7	7.29	54.5	7.38
2 gr	109	48.5	7.33	55.5	7.39
50	111	56.5	7.40	57.0	7.40
52	116	56.2	7.40	56.7	7.40
70	126	48.2	7.33	49.6	7.34
3	136	37.9	7.22	37.8	7.22
60	140	58.3	7.42	62.0	7.44

The results of these experiments are presented in Table 2. The maternal alkali reserve given is that determined on the first sample of blood drawn from the external jugular whilst the ewe was standing. The actual dissociation curves of two cases, No. 54 and 52, are illustrated in Figures 4 and 5. If these data represent the normal relations *in utero,* they indicate that the fetal alkali reserve is about equal to or less than that of the maternal blood. In no case was it higher. They further indicate that the fetal reserve

is lower in early stages of gestation—in the period of implantation—relative to the maternal than it is in the last month of gestation though the data would not warrant a final conclusion.

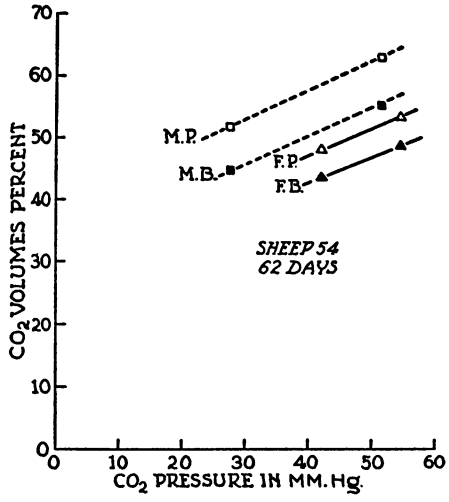

FIG. 4. Fetal and maternal carbon dioxide dissociation curves of oxygenated whole blood and true plasma. Sheep No. 54, 62 days.

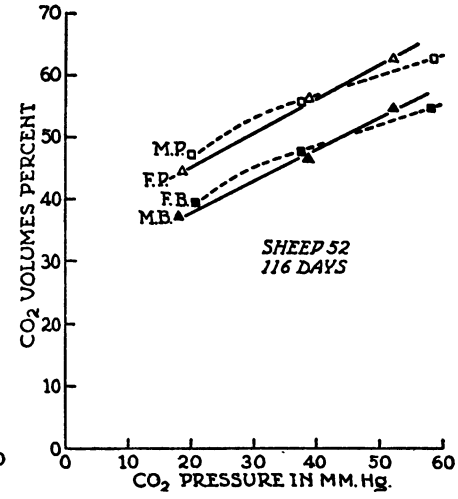

FIG. 5. Fetal and maternal carbon dioxide dissociation curves of oxygenated whole blood and true plasma. Sheep No. 52, 116 days.

SUMMARY AND CONCLUSIONS

The observations presented here appear to justify two conclusions: (1) that the alkali reserve of the ewe is not diminished during pregnancy below the range established for normal non-gravid individuals, and (2) the reserve of the fetal plasma is equal to or less than that of the ewe. These results suggest that the plasma of the sheep is not affected by pregnancy in the same fashion as reported by Keys in the goat in which the maternal alkali reserve appears to be lower with respect to that of non-gravid animals and fetal kids. Where these differences in the response of the pregnant sheep and goat have their origin is a matter of speculation and a subject for future research.

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