FRONTISPIECE

SOME EFFECTS OF SODIUM DEPLETION IN PREGNANT SHEEP

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ABSTRACT

Some effects of sodium depletion were investigated in sixteen sheep at different stages of pregnancy ranging from 55 to 139 days. The sheep were acutely depleted of sodium by draining the saliva from one parotid gland for a period of six days. Along with each depleted ewe, similar observations were made on a similar non-depleted ewe.

The effects of sodium depletion on the pregnant ewes were similar to those reported in non-pregnant sheep.

The results indicate that acute sodium depletion of pregnant sheep causes a deficiency of sodium in the foetuses. The foetal plasma and the amniotic fluid had significantly lower sodium levels and the allantoic fluid volume was significantly greater in the depleted animals.

The evidence indicates that foetal urine is a major source of amniotic fluid in older sheep foetuses.

It is suggested that a sodium deficient foetus has adjustment mechanisms similar to those of a sodium deficient adult.

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INTRODUCTION

The primordial sea in which life is believed to have originated several billion years ago was of a lower total salinity and a much higher potassium concentration than today's oceans. Whether this is related to the universally high intracellular concentration of potassium today is obscure but the fact remains that in the present day organisms potassium is fairly well confined to the intracellular space, whereas sodium remains principally in the extracellular space. With the progress of evolution and the ascent of organisms to dry land, the regulation of this extracellular fluid, or internal environment, became of prime importance to animals for freedom and independence of existence. The constancy in composition of the extracellular fluid within each vertebrate species is remarkable and is determined largely by what the kidney retains - provided there is always a surplus.

Practically all of the sodium in the animal body enters in the form of sodium chloride and, for the most part, functions and is excreted in its ionized form. Sodium chloride helps to maintain the acid-base balance of the body and is largely responsible for the total osmotic pressure of the extracellular fluids. Sodium also has an important function in muscle contraction and the propagation

of nerve impulses. There is a regular dietary need for sodium because of limited storage and rapid excretion of all excesses. On the other hand, the animal has a remarkable ability to conserve sodium when the supply is inadequate. It has been stated that aldosterone, a steroid hormone secreted by the adrenal cortex, is the most important sodium retaining hormone.

The immediate effect of a depletion of sodium from the body is a fall in the osmotic pressure of the extracellular fluid due to reduction in the sodium and chloride concentrations. This gives rise to an outward movement of water from the extracellular space into the cellular space. Also the kidneys excrete water leading to a diminution of the extracellular fluid volume; this is secondary dehydration. The excretion of sodium in the urine virtually stops. The loss of plasma water gives rise to a haemoconcentration, the haematocrit value and the protein concentration in the blood increase, the blood pressure falls, which can in severe cases, lead to circulatory failure. The clinical symptoms of dehydration are anorexia, muscular weakness, loss of weight, dryness of mucous membranes and absence of thirst. When water intake is adequate, the urine volume is normal.

In recent years, tracer techniques have been successfully used to study the exchange of water and electrolytes

across the placenta, and to study the rate of exchange of sodium, potassium and water of the amniotic fluid. It is found that the amount of sodium, potassium and water supplied to the foetus across the placenta is several times greater than the amount actually incorporated into the growing tissues and that most of it is returned to the maternal circulation. Similarly, it was observed that the sodium, potassium and water of the amniotic fluid are replaced at a rapid rate.

The ratio of the quantity of a substance supplied to the foetus from the maternal plasma to the amount of the substance retained by the foetus in its growth has been called the "safety factor". This, it is believed, assures an adequate supply of these substances to the growing foetus. The doctrine that the foetus leads a parasitic life inside the mother and, when there is a shortage of nutrients, claims priority over the mother has limitations on general grounds, if only for the reason that the mother should remain alive for the development of its foetus. It was observed that sheep on a low plane of nutrition during the last third of pregnancy had lighter lambs than those on a high plane of nutrition.

The effects of acute sodium deficiency on pregnant animals and their foetuses has been the subject of only few

investigations. Kirskey et al (1962) observed that a sodium deficient diet fed to pregnant rats did not have any significant effects on the foetuses or amniotic fluid, even though the mothers were showing the effects of sodium depletion. Winkler et al (1962) report that the foetal plasma sodium showed corresponding changes to increases and decreases in the sodium concentration of maternal plasma brought about by experimental methods.

The present experiment was undertaken to study some effects of sodium depletion on pregnant sheep and to observe some of the depletion effects on the foetuses and foetal fluids. The observations were made at different stages of gestation ranging from 55 to 139 days. The ewes were acutely depleted of sodium by draining the saliva from one parotid gland for a period of six days. Along with each depleted animal, similar observations were made on a non-depleted control animal.

In considering the effects of sodium depletion on the ewes, changes were looked for in sodium and potassium concentrations of parotid saliva and plasma, plasma volume, haematocrit levels, and body weights. To discern the effects of depletion on the foetuses, changes were looked for in the sodium and potassium concentrations of foetal plasma, foetal muscle tissue and foetal fluids; in the

volumes of foetal fluids and in foetal weights.

loss of one litre of plasma. The reduction in blood volume and increase in viscosity would lead to a fall of blood pressure and, in severe cases, to circulatory failure. A fall in systemic blood pressure due to sodium depletion was observed by Denton (1957a) in sheep.

(b) Disturbance of acid-base equilibrium occurred when there was a considerable asymmetrical loss of sodium or chloride ions.

The author stated that the harmful effects in pure salt depletion are due to reduction of blood volume and increase of blood viscosity and that any disturbance of acid-base balance is of minor importance. The author further stated that the sodium or chloride concentration of the plasma is not useful as an estimate of the salt deficit because of the adjustment mechanisms.

McCance (1937) produced salt deficiency in man by a combination of diet and sweating. There was a fall in the concentration of sodium and chloride in the serum, no change in plasma bicarbonates and no consistent change in the serum potassium level. There was a fall in the osmotic pressure of the extracellular fluid and an increase in plasma proteins. In a similar experiment McCance (1938) observed a reduction of 28 to 39 per cent in the extracellular fluid volume. Denton (1957b) observed in a sheep

that withdrawal of 1110 mEq of sodium from the body caused a 32 per cent decrease in the congo red space (plasma volume) and a 33 per cent decrease in antipyrene space (total body water). Hix, Underbjerg and Hughes (1959) observed a similar response to severe dehydration in two sheep on a diet low in sodium and stated that the dehydration was generalized and not confined to the extracellular water.

Darrow and Yannet (1935) produced NaCl deficiency in dogs, rabbits and monkeys by introducing a 5 per cent solution of glucose into the peritoneal cavity of the animals. This caused an osmotic movement of sodium, chloride and other extracellular solutes into the peritoneal cavity. Subsequent drainage of an equal amount of fluid after 4 hours resulted in permanent removal of these extracellular solutes from the body. Following the intraperitoneal injection, in all animals, signs of mild to severe dehydration developed. No urine was passed for 4 to 6 hours after the injection and in spite of the signs of dehydration the animals were not thirsty. There was a loss of extracellular water with little or no change in total body water. There was 18 to 49 per cent reduction in plasma volume and, in all cases, considerable decrease in plasma Na^+ and Cl^- levels. The proportion of red cells in the blood and the

concentration of serum proteins were increased. There was a 1.4 to 7.3 per cent increase in red cell water and a reduction in red cell protein concentration. In direct contrast to the reports of Denton (1957b) and Hix et al (1959), the authors interpreted the changes as a shift of extracellular water into intracellular space due to a decrease in the osmotic pressure of the extracellular fluid, with essentially no loss of water from the body. The authors stated that the relative osmotic pressures of the intra- and extracellular fluids seem to be the main factors governing the distribution of water in the body. Darrow and Yannet (1936) produced a more severe salt depletion in dogs and obtained similar results. In all experiments, restoration of extracellular electrolytes by intraperitoneal injection of NaCl solution, brought the concentration of serum electrolytes to approximately normal levels. Loss of appetite leading to starvation was a striking symptom of the NaCl deficiency.

Baker, Levitin and Epstein (1961) observed that rats depleted of sodium and rendered hyponatremic by peritoneal dialysis developed severe, reversible impairment of renal concentrating ability, resulting in increased urinary losses of water, rapid weight loss and eventual restoration of serum sodium concentration to normal range. Darrow and

Yannet (1936) observed that during sodium depletion in dogs, when there was a small loss of Na and Cl ions, enough water was excreted almost to restore the concentration of electrolytes in the serum.

It would seem that the immediate reaction to an acute sodium depletion is an osmotic movement of water from the extracellular space to the intracellular space, but later on, as the sodium deficiency continues, the animal excretes water and there is a loss of water from both the compartments.

Nichols and Nichols (1956) investigated the changes in the tissue composition of dogs during acute sodium depletion. They produced a pure, acute sodium deficiency in dogs by vivodialysis resulting in the removal of approximately 23 per cent of total body sodium. It was found that the extracellular fluid phase of the body contributed 70 per cent, bone mineral 25 per cent and body cells only 5 per cent of the total sodium removed. Highly vascular tissues, such as muscle and skin, had a rapid initial rate of sodium removal which declined with time as sodium stores were rapidly depleted. The bone showed a falling rate of sodium removal whereas other avascular tissues, such as tendon, showed an increasing rate of sodium removal with time. The fall in plasma sodium concentration was relatively small while there was a slight increase in the

potassium concentration. The authors concluded that bone constituted a true reservoir of sodium which was rapidly available in the face of acute demand. Kirskey, Pike and Callahan (1962) observed a 10 per cent decrease in the bone sodium of pregnant rats on a low sodium diet. However, Baker et al (1961) observed that in rats rendered hyponatremic, the contribution of bone sodium to replacement of sodium deficits was negligible.

Denton (1957b) stated that the sheep rumen normally contains a pool of 100-500 mEq of Na^+ and that the salivary response to Na^+ depletion can probably transform the largely Na^+ digestive pool to a K^+ pool so that the Na^+ becomes available for the extracellular fluid. In one Na^+ depleted sheep the $Na^+:K^+$ ratio of the rumen fluid was 0.21 whereas in a normal animal it was 4.0.

Denton, Wynn, McDonald and Simon (1951) observed in man and sheep that when Na^+ was subtracted from the body in excess of Cl^- relative to the extracellular ratio of these two ions, the ratio Cl^-/Na^+ in the extracellular fluid remained constant. It was found that this was achieved by a selective excretion of Cl^- on the part of the kidney. This mechanism seemed to operate even when this caused a depletion of the total amount of extracellular ions and great decreases in plasma volume. Denton (1957b) also

observed increased renal excretion of Cl^- during Na^+ depletion in sheep. The author stated that this selective excretion of Cl^- during sodium loss was a very important mechanism in abating acidosis. However, Nichols and Nichols (1956) stated that in dogs a severe depletion of sodium, resulted in a severe uncompensated metabolic acidosis, whereas a moderate depletion did not significantly affect blood pH.

The different response in the dog may be due to its normally acidic urine excretion which in sheep is normally alkaline.

Denton (1958) fed an artificial diet, containing a low level of potassium, to sheep with permanent unilateral parotid fistulae. Provided there was no Na^+ deficit, the animals could be maintained on this diet for 1-2 months. The K^+ intake was less than the maximum parotid K^+ output observed under conditions of Na^+ depletion. It was observed that Na^+ depletion in the absence of an adequate intake of K^+ had a severe impact on the sheep's condition, and that an experiment could not be continued longer than 4 to 6 days. There was a decrease in the plasma K^+ level as against an increase in the plasma K^+ level of sodium depleted animals receiving an adequate intake of K^+. The restriction of K^+ intake was reflected in an active urinary

conservation of K+. The fall in whole blood pH was greater during Na+ depletion when the K+ intake was low than when the K+ intake was adequate.

Effect of Sodium and Potassium Depletion in Pregnant Animals.

The general effects of sodium deficiency have been long known and the patterns are well established. In the last few years some investigations have been carried out to study the effects of sodium deficiency in pregnant rats and to observe if these effects are in any way reflected in the foetuses and foetal fluids.

Kirskey, Pike and Callahan (1962) investigated the effects of high and low sodium intakes during pregnancy in the rat. On the day of mating, rats were divided into three groups and fed three different levels of sodium: low, high and normal. Three groups of non-pregnant rats were maintained on similar rations. The experiment was terminated on the twenty second day of gestation. The group fed low sodium generally exhibited the typical effects of sodium deficiency. A significant decrease in sodium and increase in potassium levels of plasma occurred in pregnant animals fed the low sodium diet. The pregnant groups on low sodium showed an approximate 35 per cent decrease in sodium and 4

per cent increase in potassium concentrations of muscle. These animals also had significant increases in muscle moisture. Bone and brain sodium concentrations were significantly lower in the pregnant groups receiving the low sodium intake. Although the average sodium concentrations of foetal plasma and amniotic fluid were slightly decreased in the low sodium group and slightly increased in the high sodium group when compared with the average control values, the differences were not statistically significant. The sodium or potassium levels of total foetuses were not significantly influenced by the maternal sodium intake. There was a significant increase in the foetal moisture of animals on low sodium intake which the authors attributed to foetal immaturity. From the fact that non-pregnant rats fed the same low level of sodium as the pregnant rats, did not show any observable effects on sodium and potassium concentrations of tissues and fluids, the authors concluded that the marked electrolyte change in the pregnant rats was an attempt, apparently successful, to maintain the normalcy of the foetuses.

Winkler, Theil and Goetze (1962) studied the changes in the foetal serum and amniotic fluid after causing changes in the sodium concentration of maternal serum in pregnant rats. Intraperitoneal dialysis with hypotonic sodium

chloride solution produced a decrease of the sodium concentration in the serum of both pregnant rats and their foetuses. After 5 hours maternal serum sodium returned to normal whereas foetal serum sodium remained low. Intravenous injection with a 20 per cent sodium chloride solution of the pregnant rats caused a temporary increase and subsequent rapid decrease of the sodium level in the serum. In the foetuses the increase occurred later, but the high sodium values were maintained for a longer period. In all experimental series the changes of the sodium and potassium content of the amniotic fluid were relatively insignificant.

Stewart and Welt (1961) investigated the effects on foetus of potassium depletion of rats by potassium deficient diet and by peritoneal dialysis. In all groups maternal serum and muscle potassium were found to be significantly lower than in the control groups. However, no significant difference in total foetal potassium concentrations was found between potassium depleted and control animals. Since the quantity of potassium in the extracellular fluid is so small compared to that in the whole foetus, a depressed extracellular fluid concentration would not necessarily have been reflected in the total carcass estimate. The mean dry weight of 12-13 day foetuses

of rats fed on a low potassium diet from the 1st day of gestation was similar to that of the control groups. However, the mean dry weight of 21-day foetuses of rats fed on the low potassium diet from the 1st day of gestation was significantly lower than that of the foetuses from control animals. Thus there was an absolute but not a relative deficit of potassium in the foetuses of deficient mothers. The authors did not explain the reason for the lesser foetal dry weight of the K^+ deficient mothers but interpreted the results as a sparing of the foetus by some unknown mechanism in the face of an acute potassium deficiency in the mother.

In the light of the knowledge that most of the foetal growth takes place in the later part of gestation, the results of Stewart and Welt (1961) can be interpreted as a significant reduction in the growth of the foetuses due to a potassium shortage for tissue growth.

Sodium State of the Body and Its Relation to Saliva

McDougall (1948) examined the composition of the parotid saliva of sheep. He obtained the saliva by cannulating the parotid duct and leading the saliva off through valve rubber tubing. The volume of saliva formed by a single parotid gland ranged from 930 to 1840 ml in 24 hours.

Eating and rumination caused a considerable increase in the rate of secretion. The saliva was alkaline, rich in CO_2 and the average sodium, potassium and chloride concentrations were 177 mEq/l, 8 mEq/l and 17 mEq/l respectively.

McCance (1938) observed that during salt deficiency in man, the saliva showed a fall in the sodium concentration and a rise in the potassium concentration.

Denton (1956) studied the effect of Na+ depletion on the parotid saliva of sheep. He made permanent unilateral parotid fistulae and observed that each parotid gland of the sheep produced more than a litre of saliva per day, with an average sodium concentration of 180 mEq/l. Draining the saliva from one gland caused a rapid and effective depletion of sodium from the animal body. When no supplementary sodium was fed, in all instances it was found that, by the end of the first three days of depletion there was a sodium deficiency of over 500 mEq. During depletion there was a gradual decrease in the saliva volume which, after three days of Na+ depletion, was approxmately one litre per day less than during the control period. The saliva sodium concentration fell from a normal of 180 to 60-80 mEq/l and, the concentration of potassium rose from a normal of 10 to 110-120 mEq/l. Thus due to

depletion the $Na^+:K^+$ ratio of parotid saliva fell to 0.5 from a normal of 18.0. There was a commensurate relationship between the amount of Na+ depletion and the $Na^+:K^+$ ratio of the parotid saliva. Within 48 hours of the Na+ depletion the Na+ excretion by the kidney was virtually nil and the excretion of K+ greatly reduced. The author stated that during sodium depletion the large K+ excretion in the urine, characteristic of all herbivora, was shifted to saliva.

Denton (1957b) studied thirty-seven sheep with parotid fistulae and obtained essentially similar results. It was also observed that restoration of a dietary supplement of Na+ in an amount to correct Na+ deficits, caused the parotid saliva composition to return to normal. An inadequate supplement caused only a partial return to normal. Depletion caused a reduction of 30-50 per cent in the salivary secretion rate.

Denton (1958) observed that during Na+ depletion on low K+ intake the saliva volume showed a greater decrease while the fall in saliva $Na^+:K^+$ ratio was less than when the K^+ intake was adequate.

The mechanism of regulation of the salivary response in sheep to sodium depletion has been investigated by Goding and Denton (1956), Denton (1957a) and Goding and

Denton (1959). It is believed that there are two processes involved in the secretion response to Na^+ depletion of the normal sheep's parotid glands. The more important of the two is the increased secretion of electrolyte active hormone by the adrenal gland in response to sodium depletion. This hormone acts on the parotid gland to lower the salivary $Na^+:K^+$ ratio. It was observed that the hormone requirement increased as the sodium depletion became more acute. The second is a local parotid factor associated with secretion rate which, in a sodium depleted sheep, lowers the salivary $Na^+:K^+$ ratio. This factor is active only in sodium depleted sheep and seems to be responsible for a positive correlation between secretion rate and $Na^+:K^+$ ratio of the saliva.

Foetal Fluids and Their Origin: Placental Transfer

Most of the early investigations on foetal fluids have been reviewed by Needham (1931). More recently Malan, Malan and Curson (1937) presented extensive data on the volume and composition of foetal fluids in the sheep at different stages of gestation. They pointed out that there is considerable variability in the amounts of both amniotic and allantoic fluids. It was observed that in early pregnancy the volume of amniotic fluid

was considerably the smaller but increased rapidly to overtake the allantoic fluid volume by the end of third month. Towards term there was no obvious difference between the amounts of the two fluids. Both the fluids were much more abundant relative to the foetal weight during the early as compared with the late stages of gestation. Regarding the composition of foetal fluids, it was reported that the sodium concentration of the amniotic fluid was considerably in excess of its potassium concentration at all stages of gestation. In the case of allantoic fluid, the sodium concentration decreased as gestation proceeded and the potassium concentration increased to exceed the sodium concentration.

Cloete (1939) observed in sheep that the volume of amniotic fluid was greatest in the third month whereas the volume of allantoic fluid reached its peak in the last month of gestation. Arthur (1957) observed that the sheep amniotic fluid showed a great increase during the third month and a decrease towards term while the allantoic fluid accumulated slowly at three months and increased greatly from the fourth month.

Wallace (1948) observed that both the fluids increased in volume fairly regularly throughout pregnancy. The author stated that in both the fluids there were con-

siderable variations which did not appear to be inter-related, or connected with the size of the foetus.

McDougall (1949) examined the composition of foetal fluids of sheep at different stages of gestation. The allantoic fluid was variable in composition and showed an increase in potassium concentration as gestation proceeded. The amniotic fluid had a much more constant composition. Sodium concentration in the amniotic fluid was higher than in allantoic fluid but the amniotic potassium concentration fell within the range of values for the allantoic fluid. The mean sodium and potassium concentrations of the amniotic fluid were 124.8 mEq/l and 13.1 mEq/l respectively. After comparing the composition of foetal fluids with that of sheep serum and foetal serum, the author suggested that the amniotic fluid arises as a transudate of the maternal serum, and the allantoic fluid from the amniotic fluid through the intervention of foetus.

Meschia, Battaglia and Barron (1957) determined the osmotic pressure of foetal and maternal plasma in sheep and goats, by the freezing point technique, from 49 days of gestation to term. It was found that in all cases the osmotic pressure of foetal plasma was equal to or slightly below that of the maternal plasma.

Alexander, Nixon, Widdas and Wohlzogen(1958a) com-

pared the composition of foetal fluids with that of maternal and foetal plasma and urine during the latter half of pregnancy in sheep. The osmotic pressures of foetal and maternal plasma were found to be similar at different stages of gestation. No consistent trend was observed in the sodium concentration in either of the foetal fluids but that in the amniotic fluid was higher than that in the allantoic fluid. The mean sodium concentration of amniotic fluid was 115 mEq/l with a range of 85-146 and that of the allantoic fluid was 70 mEq/l with a range of 32-131. In general the potassium concentration of amniotic fluid (mean 10.6, range 4.7-30.0 mEq/l) was found to decline as term is approached, while the converse was suggested by the values found for allantoic fluid (mean 10.2, range 1-33 mEq/l). The mean maternal and foetal plasma sodium concentrations were 160 and 152 mEq/l respectively, while the potassium concentrations were 6.0 and 6.7 mEq/l respectively. In the foetal urine, the sodium concentration declined with increasing foetal age while there was a suggestion of a rise in the potassium concentration. The authors suggested that foetal urine is the probable source of allantoic fluid and that the amniotic fluid is derived from the foetal or maternal plasma, particularly in the younger foetuses. In older foetuses,

amniotic fluid is modifed by the addition of foetal urine and other foetal secretions and by removal by foetal swallowing. Alexander, Nixon, Widdas and Wohlzogen (1958b) observed that the sheep foetus produced large quantities of urine which was in excess of adult resting values. That an equal quantity of amniotic fluid can be absorbed by the sheep foetal gut has been reported by Wright and Nixon (1961).

Danforth and Hull (1958) examined the microscopic anatomy of the amnion and indicated that the epithelial cells of the amnion have secretory activity and may be the major source of amniotic fluid.

The use of isotope tracer techniques in the study of foetal fluids and placental transfer has added some new concepts to the question of the origin of foetal fluids and the supply of material to the foetus across the placenta.

Vosburgh, Flexner, Cowie, Hellman, Proctor and Wilde (1948) using D_2O and radioactive sodium, measured the rate of renewal in women of the water and sodium of amniotic fluid. It was observed that the water of the fluid was completely replaced on the average every 2.9 hours and the sodium turned over every 14.5 hours. From the fact that in the earlier stages of pregnancy a volume of water equal

to that of the foetus flows in and out of the amniotic sac in an hour, the authors concluded that the foetal urine alone cannot possibly account for this relatively large volume of fluid.

Neslen, Hunter and Plentl (1954) used tracer techniques to determine the rate of exchange of sodium, potassium, and water between amniotic fluid and maternal system in women. The number of mEq of sodium exchanged per minute was 35 times greater than the number of mEq of potassium. The ratio of these exchange rates was about the same as the ratio of their respective concentrations in the amniotic fluid. The exchange rate of water was five times greater than that for the electrolytes. The rates of transfer of sodium ions to and from the amniotic fluid were equal. The authors stated that water and electrolytes of the amniotic fluid are in dynamic equilibrium with maternal plasma, each exchanging at its own characteristic rate. Friedman, Gray, Hutchinson and Plentl (1959) observed in pregnant monkeys at term that 75 per cent of the water transferred from the amniotic fluid to the mother was transmitted via the foetus.

Hutchinson, Gray, Plentl, Alvarez, Caldeyro-barcia, Kaplan and Lind (1959) reported that the role of the human foetus in the transfer of water from amniotic

fluid to mother increases with gestation and at term about 40 per cent of the water transfer from the amniotic fluid to mother is accomplished through the intermedium of the foetus. It was also observed that the rate of renewal of water of the amniotic fluid and the rate of exchange of water between the mother and the foetus increased with age of foetus until delivery.

Hellman, Flexner, Wilde, Vosburgh and Proctor (1948) investigated the permeability of the human placenta to water. From the 14th week of gestation to term there was a five fold increase in the transfer rate of water per unit weight of placenta. Fourteen and 31 week old human foetuses received across the placenta 700 times and 3800 times respectively as much water as is incorporated in the growing tissues.

Flexner, Cowie, Hellman, Wilde and Vosburgh (1948) observed that the permeability of placenta to sodium increased about 70 times from 9th week to end of gestation. The human foetus received across the placenta at the 12th week of pregnancy 160 times, and at the 40th week 1100 times as much sodium as is incorporated in the growing tissue. The ratio of the quantity of a substance supplied to the foetus from the maternal plasma to the amount of that substance retained by the foetus in its growth has been called the

"safety factor".

Flexner and Gellhorn (1942) studied the comparative physiology of placental transfer of sodium in six animals representing the four morphologic types of placenta: epitheliochorial, syndesmochorial, endotheliochorial and haemochorial. The rate of transfer of sodium increased in each animal as gestation proceeded until just before term, when there was a sharp decrease. Variations in the rate of transfer across the placentas were correlated with morphological changes in structure. The rates of transfer of sodium across unit weight of the four types of placentas were found to depend upon the morphologic structure of the placenta: The fewer the number of tissue layers between maternal and foetal circulation, the greater the rate of transfer. A correlation was also found to exist between the supply of sodium transferred to a unit weight of foetus and the rate at which that unit weight of foetus was growing.

Villee (1960) summarised the proceedings of the conference on the source of amniotic fluid. Infants born with bilateral renal agenesis have no amniotic fluid. In contrast, atresia of the oesophagus is almost always associated with an excessive amount of amniotic fluid. Apparently under normal conditions the foetus swallows amniotic fluid

and then excretes it through the kidneys. The amniotic fluid may be derived from foetal urine or from amniotic epithelium or from both. That the lungs may contribute significantly to the amniotic fluid is indicated by the finding that when the neck of the foetal rabbit is tied, fluid accumulates in its lungs. The results of experiments with deuterium and tritium show that there is an extremely rapid exchange of water molecules between the maternal circulation, the foetal circulation, and the amniotic fluid. The total amount that is swallowed and excreted by the foetus is a very small fraction of the amount that undergoes molecular exchange.

After examining much of the experimental work, it is evident that many factors contribute to the origin and regulation of the amniotic fluid. It is not clear whether the rapid exchange of the water and electrolytes of the amniotic fluid has any bearing on the regulation of the volume and composition of the amniotic fluid. It would seem that the foetal kidneys, lungs and the membranes all contribute to the amniotic fluid, some of which is swallowed by the foetus. The nature of the allantoic fluid suggests the foetal urine as its principal source but here also other factors may be involved.

EXPERIMENTAL PROCEDURE

Experimental Animals

Forty-seven ewes were selected from a flock of sixty-seven purchased from a ranch. The animals were crossbred, but predominantly Suffolk. Selection was for uniformity in characteristics, and for animals which had lambed at least once. Extremes in weight and age were avoided. The ewes ranged in age from 2-5 years.

Breeding of Animals

Early in the breeding season, a Suffolk ram was introduced into the pen for breeding the ewes. A crayon marker was strapped onto the brisket of the ram. The colour markings on the ewes were examined every day to determine the date of service and the colour of the marker was changed every fortnight. For each ewe the last service was taken as the date of breeding. When thirty-six animals were considered pregnant, they were sheared and moved into the heated part of the barn.

Design of Experiment

Thirty-two ewes were allotted into sixteen pairs in such a way that both the animals in a pair had the same

date of breeding. In pairing, all one-day differences in the dates of breeding were ignored. The remaining four animals were kept as stand-by for replacements. The two animals in each pair were randomly allotted into a depletion animal and a control animal. The sixteen pairs of ewes were allotted caesarian operation dates on a schedule wherein operations would be performed at different stages of pregnancy between 55 and 139 days, at approximately 5 day intervals. However, caesarian section could only be performed on two days a week and so there were some variations in spacing.

Diet

The ewes were group-fed on the following ration throughout the experiment, except during sodium depletion when no supplementary salt was allowed.

Bromgrass-alfalfa hay (3:1)	2.5 lbs per head/day
Barley-oat grain mixture (3:1)	1.0 lb per head/day
Iodised salt	2.0 gm per head/day

A block of iodized rock salt was placed in the pen and water was provided *ad libitum*.

Procedure of Sampling

Sodium depletion of the animal in each pair was

obtained by draining the saliva from the right parotid gland for a period of six days. The following determinations were made or samples were collected on the day of the commencement of sodium depletion and on the day prior to Caesarian sections.

1. Weight of the animal.
2. Parotid saliva.
3. Heparinised blood for haematocrit estimation.
4. Heparinised blood for plasma separation.
5. Plasma volume (T-1824 space).

Parallel with the depletion animal, similar procedure was followed with the non-depleted control animal.

During depletion the depleting animal was placed in a separate pen and allowed the same ration, less the salt, while the control animal remained in its original pen and was fed the regular ration. Daily samples of parotid saliva were collected from the depleting animal. The control animal was fasted for one day before operation.

On the day of operation both the animals of the pair were moved to the surgery and caesarian sections performed.

The same procedure was followed with all the pairs throughout the experiment.

Parotid Saliva Collection and Draining Technique

Polyethylene tubing of I.D. 0.045" and O.D. 0.062" was used for collecting and draining the saliva. The ewe was placed on its side on a low table and an assistant held the mouth slightly open and pointing upwards. A laryngoscope was passed along the buccal side of the cheek and the opening of the parotid duct located. With the help of a steel stilette one end of an approximately 8" long piece of the polyethylene tubing was passed into the duct. The stilette was withdrawn slowly and about two inches of the tube was pushed into the duct. Clear, watery saliva started flowing and was collected in small glass vials. Generally the procedure took a few minutes.

In the case of animals that were to be depleted, after introducing one end of the polyethylene tube into the salivary duct, a 12 gauge hypodermic needle was inserted through the cheek so as to emerge inside the mouth about one-quarter of an inch anterior to the opening of the parotid duct. The free end of the tube was then passed through the lumen of the needle, brought outside, and the needle withdrawn. The tube was fixed to the cheek at its point of emergence by tying it to a superficial skin suture. Part of the tube was then snipped off so as to leave approximately two inches projecting from the cheek. A free flow

was established and the saliva effectively drained (Frontispiece).

Plasma Volume Determination Technique

In general the procedure outlined by Gregerson and Stewart (1939), was followed. A 1.5 per cent solution of T-1824 in distilled water was prepared, filtered, autoclaved and stored in 250 ml rubber stoppered bottles. All other equipment was sterilized before use. The animal was restrained and a 14 gauge hypodermic needle inserted into a jugular vein. After collecting a sample of blood for serum separation and two heparinised sampled for haematocrit determination and plasma separation, a 10 inch long polyethylene tube of I.D. 0.034" and O.D. 0.060" was introduced into the vein through the lumen of the needle and the needle withdrawn. The tube was flushed with sterile normal saline and the free end plugged. The tube was attached to the neck of the animal with adhesive tape. Then with an accurately calibrated syringe exactly 5 ml of the T-1824 solution was injected into the other jugular vein using an 18 gauge needle and the time of injection noted. Starting twenty minutes after the injection of the dye seven blood samples were collected through the polyethylene tube at

approximately 15 minute intervals. The time of collection of each sample was noted to the nearest minute. To prevent the blood from clotting in the tube, the tube was flushed with normal saline and plugged after each sample collection. All of these samples, including the sample of undyed blood, were allowed to clot and centrifuged at 2,500 r.p.m. for 30 minutes. The supernatant serum was separated and kept frozen in glass vials until analysed.

The concentration of dye in the serum samples was determined in a Coleman Nepho-colorimeter, using a Ca2l-205 filter which had a peak absorbance at approximately 625 mu. In preliminary experiments using known concentrations of the dye in sheep serum, a straight line relationship was found to exist between dye concentration and absorbance. For reading the unknown dye-tinged samples, 5 ml of distilled water was added to 1.0 ml of the unknown serum and read against a serum blank prepared in a similar manner with preinjection serum from the same sheep. The absorbances of T-1824 in the samples were plotted against time. The curve usually was approximately a straight line which was extrapolated to zero time. The dye concentration at zero time is theoretically equal to the true value if all the dye injected was immediately and uniformly distributed in blood. From this value, D_p(x6 for the

dilution factor), and from the absorbance of the standard 1.5 per cent T-1824 in known dilution (1-6000) D_k, the plasma volume was calculated as follows:

$$\text{Plasma volume ml} = \frac{D_k \times 6000 \times \text{ml dye injected}}{D_p \times 6}$$

Caesarian Section and Foetal Samples Collection Technique

Intravenous Nembutal was employed as anaesthetic. The ewe was placed on its back and the abdominal region prepared for surgery. The uterus was exposed and brought to the surface through a mid-line incision along the linea alba from just in front of the mammary glands to the umbilicus. The uterus was incised, and the allanto-chorionic membrane exposed. In the earlier stages of gestation the allantoic fluid seemed to be under pressure, as severing this membrane caused the fluid to flow out. The allantoic fluid was collected in a beaker and the intact amniotic sac with the foetus inside was lowered into another beaker. Samples of amniotic and allantoic fluids were collected without contamination. The umbilical cord was exposed and a sample of foetal blood from an umbilical artery was collected in a heparinised syringe. In the advanced stages of gestation little difficulty was experienced in exposing

both the allantoic as well as amniotic sacs intact, which were lowered into two trays (Figure 1). After collecting the necessary samples, the umbilical cord was ligated and severed. The volumes of amniotic and allantoic fluids were measured and the foetus weighed. The foetus and all other samples were kept frozen until analysis. In the case of twins, the same procedure was followed with each foetus.

Analytical Methods

Haematocrit was estimated by centrifuging the heparinised blood in Wintrobe haemotocrit tubes at 2,500 r.p.m. for 30 minutes.

Plasma was separated by centrifuging the heparinised blood in test tubes at 2,500 r.p.m. for 20 minutes.

Samples of saliva, maternal and foetal plasma, foetal fluids and foetal muscle tissue were analysed for sodium and potassium by Flame photometry, using an internal standard method described by Berry, Chappel and Barnes (1946). An "Advance Flame Photometer" was used in which lithium at 300 p.p.m. was used as the internal standard.

For sodium and potassium analysis all the fluid samples were diluted with de-ionised water, so as to fall within the reading range of the flame photometer, and

AMNIOTIC SAC

CONTAINING FOETUS

ALLANTOIC

SAC

FIGURE 1

Foetal Samples Collection.

analysed directly.

For the analysis of muscle tissue, approximately 1.0 gm of gastrocnemius muscle was removed, blotted, weighed accurately and digested in micro-kjeldahl flasks. The organic matter was partially digested with 4 ml of nitric acid after which one millilitre of perchloric acid was added and the heat increased to complete the digestion and drive off the nitric acid. The digest was cooled and made up to 50 ml with de-ionised water. Sodium content was analysed directly from this solution and further dilutions were made for potassium determinations.

Statistical Methods

Statistical methods used on the maternal data were analysis of variance as described by Snedecor (1956) and Duncan's multiple range test as outlined by Steel and Torrie (1960). For this purpose the four sets of data representing before depletion and after depletion values in the depleted group and the parallel, initial and final, values from the control group were dealt with as four treatments.

The data from foetuses and foetal fluids of the control group were compared with that of the depleted group by covariance analysis as outlined by Snedecor (1956).

RESULTS

In reporting the results and in discussion the term "sodium depletion" has been used to mean draining the parotid saliva from one gland in the absence of any supplementary intake of sodium.

One depletion animal allotted caesarian operation on the 66th day of gestation, died on the third day of depletion; post-mortem examination revealed abscesses in the liver. No substitution could be made for this animal. Another depletion animal, allotted operation on the 139th day of gestation, aborted a dead foetus on the morning of the operation day. The control animal of this pair died but was replaced by another from the spare animals. Another control animal allotted caesarian operation on the 94th day of gestation proved to be not pregnant, and no replacement could be made.

All the data obtained and used in statistical analysis are presented in Tables III to XIII in the Appendix.

I. Maternal Changes

General Effects

During the first two days of depletion all animals

were eating well, but later on the feed intake was considerably reduced and by the end of depletion most animals had completely stopped eating. The water intake was also comparatively low. The animals were generally dull and disinclined to move.

Saliva Changes.

In most of the depleting ewes the sodium concentration decreased steadily and the potassium concentration increased steadily in the daily saliva samples during the six days (Table XII). In some individuals there were marked day to day fluctuations. The mean daily concentration of sodium declined linearly and was associated with a similar linear increase in the potassium during the six days of depletion (Figure 2). However, the compensation was not complete and the total concentrations of cations ($Na^+ + K^+$) decreased. Sodium depletion caused a highly significant change ($P < .01$) in the sodium and potassium levels of saliva. The sodium concentration decreased from an initial mean level of 172.6 mEq/l to 38.2 mEq/l after depletion while the potassium concentration increased from an initial mean level of 5.1 mEq/l to 95.8 mEq/l after depletion (Table I). The $Na^+:K^+$ ratio of saliva fell from an initial value of 33.8 to 0.4 after depletion.

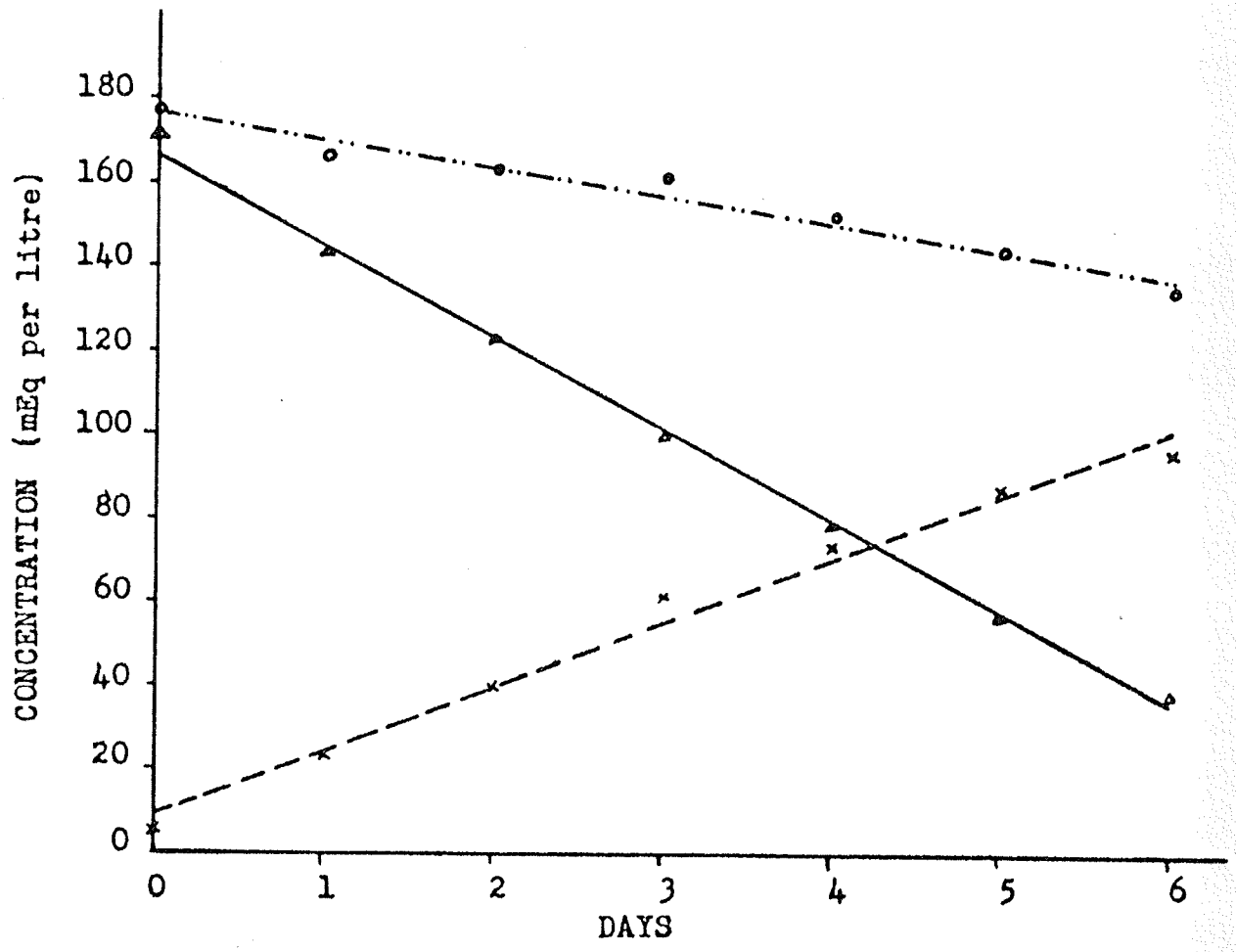

FIGURE 2

REGRESSIONS OF SODIUM, POTASSIUM AND TOTAL CATION (Na+K) CONCENTRATIONS IN THE DAILY SAMPLES OF SALIVA ON DAYS OF DEPLETION

Although no measurements were made of the daily volume of parotid saliva secreted, a decreased rate of flow was suggested by the increasing length of time required to collect the necessary volume of saliva sample from the depleting animals.

TABLE I. Summary of Maternal Data Showing Means and Standard Errors of the Initial and Final Values From the Control and Depletion Groups.

	Control		Depletion	
	Initial	Final	Initial	Final
Saliva Sodium mEq/l	171.9 ±2.0	173.9 ±1.9	172.6 ±1.9	38.2±12.3
Saliva potassium mEq/l	4.8 ±0.12	6.3 ±1.46	5.1 ±0.24	95.8±10.0
Plasma sodium mEq/l	148.8 ±0.9	148.5 ±0.8	150.0 ±0.6	135.2± 1.4
Plasma potassium mEq/l	4.5 ±0.07	4.6 ±0.11	4.6 ±0.09	4.2± 0.11
Plasma volume litres	2.96±0.13	3.01±0.07	2.62±0.15	1.70±0.10
Haematocrit per cent	40.0 ±1.0	40.0 ±0.9	40.0 ±1.1	54.0 ±1.3
Body weight kg	67.0 ±4.9	67.0 ±4.9	66.0 ±2.5	56.0 ±2.4

Blood Changes

Although no direct measurements were made, it was observed in the depleted animals that the blood was comparatively darker in colour, highly viscous, and flowed through the collection tube very slowly. Also, during caesarian section there was virtually no haemorrhage in the depleted animals suggesting a lowered blood pressure and impaired circulatory efficiency.

Sodium depletion caused significant decreases in sodium ($P < .01$) and potassium ($P < .05$) concentrations in plasma. Due to sodium depletion there was a fall of 14.8 mEq/l in the mean plasma sodium level and a fall of 0.4 mEq/l in the mean plasma potassium level (Table I).

A comparison of the initial and final plasma volumes of the depletion group showed a highly significant decrease ($P < .01$) in the plasma volume due to depletion. A similar comparison in the control group showed no significant differences. Sodium depletion caused a mean decrease of about 35 per cent in plasma volume (Table I).

Sodium depletion caused a highly significant increase ($P < .01$) in the haematocrit levels. The mean haematocrit level increased to 54 per cent from a normal mean of 40 per cent (Table I). Assuming the circulating cell volume remained

constant during depletion such a change in the haematocrit indicates a reduction of about 43 per cent in the plasma volume.

Body Weight Changes

Sodium depletion caused a highly significant reduction ($P < .01$) in the body weights of ewes. There was an average loss of 16 per cent in the body weights of sodium depleted ewes whereas the control group maintained their weight.

II. Foetal Changes

In the depleted group four out of 14 ewes had twin foetuses while in the control group one out of 15 ewes had twin foetuses. There were differences between foetuses within twins. Therefore, in statistical analyses, in the case of foetal plasma and muscle electrolyte levels the mean, and in the case of foetal fluid electrolyte levels the weighted mean, of the two values from each ewe have been used. The weighting was based on the volumes of foetal fluids for the twins. The original data from the twin foetuses are presented in Table XIII.

Among the depleted group four ewes (sheep numbers 319, 327, 308 and 316) had dead foetuses at the time of

caesarian operation. Data from the dead foetuses and associated fluids did not show any apparent trends and fell within the range of values from the live foetuses. Similarly no consistent trends in the values were apparent in relation to the sex of foetuses. Therefore, all the data were included in statistical analyses.

Statistical procedure used was analysis of covariance by which the regression of each factor on the age of foetus from the control group was compared with that of the depleted group. Also, the mean values from the two groups were compared by adjusting the means to a common age. Results of the statistical analyses are summarised in Table II.

Sodium and Potassium in Foetal Muscle

In both the control and the depleted groups foetal muscle sodium concentration showed a highly significant decrease ($P < .01$) and the potassium concentration a highly significant increase ($P < .01$) with increasing age of the foetus. Neither sodium nor potassium levels of the foetal muscle showed any significant differences due to depletion between either regressions or adjusted means.

Sodium and Potassium in Foetal Plasma

Satisfactory foetal plasma samples were obtained from only eight animals in the depleted group. There were no

TABLE II. Summary of Foetal Data Showing for Each Factor the Adjusted Means, Coefficients of Regression on Age and Coefficients of Correlation with Age for the Control and Depleted Groups

	Adj. Mean		Reg. Coef.		Corr. Coef.	
	Control	Depleted	Control	Depleted	Control	Depleted
Muscle Na mEq/100 gm	7.4	7.1	-0.059 ±0.007	-0.055 ±0.012	-0.909**	-0.800*
Muscle K mEq/100 gm	5.4	5.0	0.033 ±0.005	0.032 ±0.007	.903**	.751*
Plasma Na mEq/l	143.0	137.4	0.133 ±0.025	0.162 ±0.135	.839**	.438
Plasma K mEq/l	4.7	5.0	0.004 ±0.013	-0.039 ±0.016	.088	- .713*
Amniotic Fluid Na mEq/l	124.5	110.0	-0.32 ±0.088	-0.42 ±0.179	- .707**	- .567*
Amniotic Fluid K mEq/l	8.7	8.9	-0.037 ±0.032	-0.051 ±0.028	- .311	- .466
Allantoic Fluid Na mEq/l	61.5	59.5	-0.439 ±0.309	-0.281 ±0.292	- .379	- .279
Allantoic Fluid K mEq/l	26.3	18.6	0.892 ±0.216	0.638 ±0.226	.766**	.650*
Amniotic Fluid Volume ml	660	731	9.62 ±1.17	15.68 ±3.86	.921**	.795*
Allantoic Fluid Volume ml	386	575	8.26 ±1.83	9.10 ±2.56	.770**	.801**
Foetal weight gm	1527	1311	52.9 ±8.02	52.7 ±8.73	0.885**	0.891**

** ($P < 0.01$) * ($P < 0.05$)

significant differences in the regression of plasma sodium on age between the two groups. The mean foetal plasma sodium concentrations of the control and depleted groups were 143.0 and 137.4 mEq/l respectively, and the difference was significant ($P<.05$).

There were no significant differences between the two groups in foetal plasma potassium levels.

Sodium and Potassium in Amniotic Fluid

Sodium and potassium levels of the amniotic fluid decreased with increasing age of foetus in both control and depleted groups. But only sodium concentrations were significantly correlated ($P<.05$) with age. The amniotic fluid sodium concentrations in the two groups showed no significant differences between regressions. But there was a highly significant difference ($P<.01$) between the adjusted means of the amniotic fluid sodium levels in the two groups. The adjusted mean sodium concentrations in the amniotic fluids of the control and the depleted groups were 124.5 and 110.0 mEq/l respectively. The potassium levels in the amniotic fluid did not show significant differences between control and depleted animals.

Sodium and Potassium in Allantoic Fluid

In both the groups the allantoic fluid sodium level

decreased and the potassium level increased with increasing age of foetus. But only potassium concentrations were significantly correlated ($P < .05$) with age. Neither the sodium nor the potassium concentrations of the two groups differed significantly in either regressions or in adjusted means.

Foetal Weights

Weights from single foetuses only were used in statistical analysis (Table XI). Whether expressed as a per cent of mother's initial weight or when absolute weights were used, there were no significant differences between the foetal weights of the two groups. However, the adjusted mean weight of the depleted group foetuses was 216 gm lower than that of the control group foetuses (Table II).

Volumes of Amniotic and Allantoic Fluids

Data of foetal fluids from single foetuses only were used in statistical analyses. Also, volumes of amniotic and allantoic fluids from all the ewes, expressed as a per cent of foetal weight, were examined statistically with similar conclusions. The volumes of amniotic fluid did not show any significant differences between the two groups. The adjusted mean volume of amniotic fluid in the depleted group was 71 ml in excess of the adjusted mean volume in the

control group. The regressions of allantoic fluid volume on age of foetus were not significantly different between the two groups. But the adjusted means were significantly different ($P < .05$). After adjusting for age, the mean volume of the allantoic fluid in the depleted group was 189 ml greater than that in the control group.

Both the fluids showed considerable variations in volume at different stages of gestation but show an approximately regular increase throughout the period of gestation studied in this experiment (Table XI). In the control group the volume of amniotic fluid exceeded that of the allantoic fluid at all stages of gestation. In the depleted group the two fluids approach each other in volume due to larger allantoic fluid volumes.

DISCUSSION

Effects on the Ewe

It was observed that with advancing sodium depletion there was a progressive loss of appetite in the depleting animals. Notwithstanding the fact that the animals were supplied with a natural diet containing high levels of potassium, because of the anorexia, the daily intake of potassium during sodium depletion proved to be lower than intended.

The parotid salivary responses to sodium depletion are in general agreement with those reported by Denton (1956), Denton (1957b) and Denton (1958). As depletion progressed, the salivary sodium losses decreased while the potassium losses increased. The increased potassium losses coupled with a decreased intake in the later stages of depletion presumably caused a certain amount of potassium depletion in these sheep. Denton (1958) observed that sodium depletion in the absence of an adequate intake of potassium caused a fall of plasma potassium level and had a severe impact on the sheep's condition. This seems to have been the case in the present experiment also in which sodium depleted sheep showed a significant fall in the plasma potassium level and lost an average 16 per cent in body weight.

The effects of sodium depletion in the present experi-

ment appeared severe, and are in general agreement with the reports of Marriott (1947), Denton (1957b), Denton (1958) and Kirskey et al (1962).

No measurements of the actual deficits of sodium were made, but on the basis of the results of Denton (1956) and Denton (1957b) it was estimated that the six-day depletion period leading to a 35 per cent reduction in plasma volume would have resulted in a depletion of over 1000 mEq of sodium from the body.

The blood changes found and the clinical observations indicate that sodium depletion caused a fall of blood pressure and a severe impairment of circulatory efficiency. This view is supported by the reports of Marriott (1947) and Denton (1957a).

Comparisons of the initial and final observations of the depletion group and with those of the control group, indicate that acute depletion of sodium was produced in the treated ewes. Thus parotid saliva drainage had the same effects in pregnant ewes as have been previously found for non-pregnant sheep.

Effects on the Foetuses and Foetal Fluids

Since most, if not all, of the exchange of material

between the pregnant animal and its foetus takes place across the placenta, the immediate effects of hyponatremia in the maternal plasma would be expected to be reflected in the foetal plasma. This was found to be the case in the present experiment in which the mean plasma sodium level of foetuses from the sodium depleted ewes was significantly lower than that of foetuses from the control ewes. Winkler et al (1962) reported similar findings in pregnant rats. Comparison of the foetal and maternal plasma sodium levels shows that a fall of approximately 15 mEq/l in the maternal plasma caused a smaller but nevertheless significant reduction of about 6.0 mEq/l in the sodium level of the foetal plasma. Kirskey et al (1962) observed that a highly significant reduction in the plasma sodium levels of 21 day pregnant rats caused a slight but nonsignificant reduction in foetal plasma sodium levels. In the present experiment the mean concentration gradient of plasma sodium, which in the normal ewes was found to exist from the mother to the foetus, was to a small degree reversed by sodium depletion of the ewe. Kirskey et al (1962) observed a similar reversal of the concentration gradient in pregnant rats on a low sodium intake. This finding indicates that some mechanism, which cannot be identified from the present study, provides

the foetal plasma sodium level some degree of independence from the maternal plasma sodium level. Possibly the mechanism responsible is related to the rapid rate of exchange of sodium between the mother and its foetus which has been called the "safety factor". It is suggested that a study of the rate of exchange of sodium between the mother and its foetus in sodium depleted animals using tracer techniques may help towards a better understanding of the processes involved.

The small but significant reduction in the maternal plasma potassium levels did not result in any detectable effects on the foetal plasma potassium levels.

The death of four foetuses observed in the sodium depleted animals is believed to have been due to hypoxia rather than to a sodium deficiency *per se* in the foetuses. It is suggested that the severe haemoconcentration in the ewes led to a decreased rate of blood flow through the maternal placenta and resulted in hypoxia of the foetuses.

The sodium and potassium levels in the amniotic fluid of the control animals were in general similar to those reported by McDougall (1949) and Alexander *et al* (1958a). Alexander *et al* (1958a) reported that the sodium concentration of the sheep foetal urine declines with increasing age of the foetus. This would suggest that if foetal urine

formed a major source of the amniotic fluid, there would be a gradual decline in the amniotic fluid sodium level with increasing age of the foetus. This was found to be the case in the present experiment in which the amniotic fluid sodium level decreased significantly with increasing age of the foetus. The progressive decrease in the amniotic fluid potassium concentration observed in this experiment also agrees with the observations of Alexander et al (1958a).

The significantly lower mean concentration of sodium in the amniotic fluid of sodium depleted animals showed, on a concentration basis, greater effects of sodium depletion than did the mean foetal plasma sodium level. This would be expected if the foetus is able to restrict sodium losses in the urine in an attempt to maintain its plasma sodium level. Alexander et al (1958b) reported that in foetal sheep, renal electrolyte reabsorption occurs in the tubules with the production of large quantities of hypotonic urine.

Winkler et al (1962) rendered pregnant rats hyponatremic for short periods and observed decreases in foetal plasma sodium levels but not in the amniotic fluid sodium levels. The probable reason for this apparently contradictory result is that there will be a time lag before the fall in the foetal plasma sodium concentration would be reflected in the amniotic fluid sodium level. The length

of the time lag will be dependent on the rate of foetal urine production. Thus with short-term depletion observable changes in amniotic fluid sodium concentrations would not be expected.

The progressive decrease in sodium and increase in potassium concentrations observed in the allantoic fluid is similar to the findings of Malan et al (1937). The sodium and potassium concentrations in the allantoic fluid do not show any statistically detectable effects of sodium depletion.

The generally regular increase observed throughout gestation in the volumes of both the foetal fluids in the control group in the present experiment is similar to the findings of Wallace (1948).

Although there were no significant differences between the foetal weights of the two groups, the adjusted mean weight of the sodium deficient foetuses was 216 gm less than that of the control foetuses. This lower weight may have been due to an outward movement of water from the depleted foetuses in response to sodium depletion. It may reasonably be assumed that due to the maternal sodium depletion, this water could not have moved back to the maternal circulation. Therefore, it would have been added to the foetal fluids. Examination of the total volume of

foetal fluids in fact showed that the mean volume of foetal fluids in the depleted group was greater by 260 ml than that in the control group. It is suggested that the sodium deficient foetus like a sodium deficient adult, responds to the effects of sodium deficiency by restricting sodium losses in the urine and by excreting water.

SUMMARY AND CONCLUSIONS

Some effects of sodium depletion were investigated in sheep at different stages of pregnancy ranging from 55 to 139 days. The sheep were acutely depleted of sodium by draining the saliva from one parotid gland for a period of six days.

Data collected indicate that sodium depletion had the following effects:

1. A significant decrease in the sodium level and a significant increase in the potassium level of the parotid saliva.
2. Significant decreases in the maternal plasma sodium and potassium levels.
3. A significant decrease in the plasma volume and a significant increase in haematocrit level.
4. Significant decreases in maternal body weight.
5. Significant decreases in the sodium levels of foetal plasma and amniotic fluid, and a significant increase in the allantoic fluid volume.

The foetal changes indicate that the foetal urine is a major source of amniotic fluid.

It is suggested that a sodium deficient foetus has adjustment mechanisms, similar to those of a sodium deficient adult.

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APPENDIX

TABLE III. Initial and Final Concentrations of Sodium and Potassium in the Parotid Saliva of Control and Depletion Ewes. (mEq per litre).

Sheep No	Days in gestation	Control				Sheep No	Days in gestation	Depletion			
		Sodium		Potassium				Sodium		Potassium	
		Initial	Final	Initial	Final			Initial	Final	Initial	Final
355	55	182.4	182.4	4.3	4.7	352	55	165.6	142.4	5.7	13.3
310	60	171.2	166.8	5.1	4.3	312	60	177.6	26.3	5.0	111.2
336	66	170.4	171.2	4.7	3.7	307	72	161.2	15.6	4.4	94.4
345	72	175.2	177.6	5.1	5.9	337	78	172.0	47.1	4.5	125.6
341	78	170.4	174.8	4.6	5.0	319	82	166.0	41.4	5.4	89.6
333	82	174.8	168.4	5.1	5.4	334	88	169.6	28.5	7.3	102.4
343	88	164.8	170.4	4.5	4.4	327	94	174.6	31.7	4.5	133.6
351	100	175.2	172.0	4.6	4.7	308	100	161.6	12.3	4.4	137.6
342	104	176.8	179.2	4.9	4.9	339	104	178.4	17.3	5.0	120.0
332	113	151.2	174.8	4.8	6.3	325	113	183.2	23.0	4.4	130.4
340	118	173.2	168.4	5.6	22.4	316	118	176.8	5.6	4.3	57.2
306	124	168.4	176.0	5.6	5.2	356	124	183.2	6.8	6.5	81.6
311	131	177.6	174.0	4.1	5.2	320	131	169.6	10.5	4.5	100.8
346	135	174.8	178.4	4.4	5.6	324	135	177.6	126.4	5.4	43.0
Mean		171.9	173.9	4.8	6.3			172.6	38.2	5.1	95.8
Standard Error		2.0	1.2	0.12	1.46			1.9	12.3	0.24	10.0

TABLE IV. Initial and Final Concentrations of Sodium and potassium in the Maternal Plasma of Control and Depletion Ewes. (mEq per litre).

Sheep No	Days in gestation	Control				Sheep No	Days in gestation	Depletion			
		Sodium		Potassium				Sodium		Potassium	
		Initial	Final	Initial	Final			Initial	Final	Initial	Final
355	55	150.4	150.4	4.2	5.0	312	60	151.2	130.4	4.7	4.4
310	60	149.2	143.6	4.7	4.7	307	72	151.6	131.2	4.5	4.2
336	66	147.2	153.0	4.5	4.2	337	78	148.4	144.8	4.5	4.0
345	72	148.4	148.0	4.8	4.5	319	82	147.2	134.6	4.6	4.8
341	78	144.8	150.4	4.4	4.5	334	88	151.6	134.0	5.1	4.9
333	82	148.0	149.2	4.2	4.0	327	94	151.6	137.6	4.4	3.7
343	88	144.4	147.2	4.5	5.0	308	100	148.8	128.0	4.5	3.8
351	100	147.2	146.0	4.2	5.0	339	104	148.4	136.4	4.7	4.3
342	104	145.2	148.4	4.8	4.9	325	113	150.4	135.2	4.1	3.9
332	113	147.2	142.6	4.3	4.5	316	118	145.2	126.4	5.1	4.3
340	118	150.8	148.0	5.0	3.8	356	124	154.0	134.0	5.1	4.3
306	124	153.6	150.8	4.8	4.6	320	131	150.4	137.6	4.6	4.6
311	131	150.0	150.0	4.4	4.8	324	135	150.0	142.0	4.1	3.7
346	135	156.4	151.2	4.3	5.0	309	139	150.8	140.0	4.2	3.8
Mean		148.8	148.5	4.5	4.6			150.0	135.2	4.6	4.2
Standard Error		0.9	0.8	0.07	0.11			0.6	1.4	0.09	0.11

TABLE V. Initial and Final Plasma Volumes (T-1824 space) of Control and Depletion Ewes (litres).

Control				Depletion			
Sheep No	Days in gestation	Initial	Final	Sheep No.	Days in gestation	Initial	Final
355	55	2.33	2.99	312	60	2.18	1.22
310	60	3.13	3.18	307	72	2.19	1.42
345	72	2.26	3.62	337	78	3.04	1.74
341	78	2.95	2.90	319	82	2.18	1.61
333	82	2.36	2.64	334	88	1.99	1.45
343	88	3.36	2.58	327	94	3.07	1.54
351	100	3.22	3.06	339	104	3.46	2.15
342	104	3.17	3.05	325	113	2.83	2.23
332	113	2.79	2.94	316	118	2.42	1.77
306	124	3.09	3.19	356	124	3.26	2.10
311	131	3.86	3.15	320	131	1.92	1.45
346	135	2.94	2.84	324	135	3.09	2.18
				309	139	2.41	1.30
Mean		2.96	3.01			2.62	1.70
Standard Error		0.13	0.07			0.15	0.10

TABLE VI. Initial and Final Haematocrit Levels of the Control and Depletion Ewes (per cent)

Control				Depletion			
Sheep No	Days in gestation	Initial	Final	Sheep No	Days in gestation	Initial	Final
355	55	38	36	312	60	36	62
310	60	44	40	307	72	37	49
336	66	36	35	337	78	43	57
345	72	42	40	319	82	43	53
341	78	46	42	334	88	38	49
333	82	36	37	327	94	46	50
343	88	40	41	308	100	39	57
351	100	35	40	339	104	39	49
342	104	40	41	325	113	38	53
332	113	39	37	316	118	33	56
340	118	34	38	356	124	38	58
306	124	44	42	320	131	35	46
311	131	40	48	324	135	42	50
346	135	39	38	309	139	46	60
Mean		40.0	40.0			40.0	54.0
Standard Error		1.0	0.9			1.1	1.3

TABLE VII. Initial and Final Body Weights of the Control and Depletion Ewes. (kg).

Control				Depletion			
Sheep No	Days in gestation	Initial	Final	Sheep No	Days in gestation	Initial	Final
355	55	59	60	352	55	61	55
310	60	69	69	312	60	64	53
336	66	60	57	307	72	51	41
345	72	63	65	337	78	73	56
341	78	60	62	319	82	65	56
333	82	58	55	334	88	45	37
343	88	60	56	327	94	71	55
351	100	75	73	308	100	63	48
342	104	64	63	339	104	75	64
332	113	70	71	325	113	79	69
340	118	70	73	316	118	72	61
306	124	72	74	356	124	78	66
311	131	77	75	320	131	56	46
346	135	82	82	324	135	70	61
-	-	-	-	309	139	70	65
Mean		67.0	67.0			66	56
Standard Error		4.9	4.9			2.5	2.4

TABLE VIII. Sodium and Potassium Concentrations in Foetal Muscle Tissue (mEq per 100 gm).

Age	Na		K	
	control	depleted	control	depleted
55	9.5	7.5	4.2	4.3
60	8.9	7.8	4.8	4.6
66	9.0	-	4.5	-
72	10.0	9.2	4.3	4.3
78	8.3	9.7	4.9	4.4
82	8.5	8.6	4.6	4.3
88	9.1	8.0	4.5	4.2
94	-	7.6	-	4.4
100	6.6	6.9	5.9	3.9
104	8.4	8.2	5.2	4.9
113	5.8	5.8	6.0	5.7
118	5.5	5.6	5.7	4.8
124	5.7	4.0	6.8	7.0
131	6.5	5.2	5.9	5.9
135	4.7	4.6	6.8	7.5
139	4.6	-	7.6	-

TABLE IX. Sodium and Potassium Concentrations in Foetal Plasma (mEq per litre).

Age	Na		K	
	control	depleted	control	depleted
60	138.0	122.8	4.2	8.0
66	138.8	-	7.6	-
72	140.4	-	4.4	-
78	144.0	148.0	3.6	6.5
82	135.6	-	3.7	-
88	138.8	127.6	3.6	3.7
100	143.2	-	4.2	-
104	140.0	145.2	3.9	4.3
113	143.6	134.6	4.1	4.2
118	146.4	-	6.9	-
124	148.2	141.6	4.8	4.5
131	145.6	132.0	4.1	4.0
135	145.6	149.6	5.5	4.9
139	150.8	-	5.0	-

TABLE X. Sodium and Potassium Concentrations in the Amniotic and Allantoic Fluids (mEq per litre).

	Amniotic Fluid				Allantoic Fluid			
	Na		K		Na		K	
Age	control	depleted	control	depleted	control	depleted	control	depleted
55	134.8	130.2	15.0	10.5	27.9	53.0	2.2	2.3
60	132.4	122.0	8.4	14.9	91.6	40.1	19.5	3.8
66	133.6	-	9.8	-	88.6	-	1.3	-
72	133.6	131.6	13.3	8.6	63.2	57.6	1.5	2.6
78	136.8	93.8	7.8	5.8	65.2	104.7	15.2	10.0
82	130.4	128.8	5.9	7.1	78.2	102.0	0.2	5.2
88	134.0	125.2	9.4	13.5	45.2	48.4	1.1	1.9
94	-	126.8	-	7.9	-	86.6	-	11.3
100	118.4	85.2	6.6	12.4	109.5	-	12.9	-
104	127.6	126.0	6.4	7.5	116.0	45.5	10.5	10.2
113	123.6	80.3	4.7	6.5	-	52.3	-	14.2
118	117.2	90.0	7.2	7.6	47.2	68.2	19.2	14.7
124	92.4	95.6	7.1	8.5	31.4	9.6	64.4	82.4
131	119.6	93.6	5.0	7.8	72.2	70.0	23.8	12.5
135	101.6	114.8	15.5	6.6	9.4	35.0	108.0	70.4
139	127.2	-	7.7	-	15.0	-	87.2	-

TABLE XI. Foetal Weights (gm) and Amniotic and Allantoic Fluid Volumes (ml) of Single Foetuses.

Age	Foetal weight		Amniotic fluid volume		Allantoic fluid volume	
	control	depleted	control	depleted	control	depleted
55	43	-	190	-	40	-
60	354	75	515	240	100	335
66	104	-	345	-	315	-
72	70	49	240	180	60	150
78	269	-	400	-	115	-
82	423	338	560	750	375	570
88	242	90	650	395	100	300
94	-	810	-	740	-	775
100	1195	1033	725	775	550	475
104	522	-	600	-	200	-
113	2020	-	950	-	850	-
118	2725	2900	980	1010	610	1000
124	-	2830	-	-	-	-
131	2343	1935	835	820	410	740
135	4712	4479	1086	1794	900	920
139	4950	-	1037	-	665	-

TABLE XII. Daily Concentration of Sodium and Potassium in the Parotid Saliva of Sheep During Sodium Depletion (mEq per litre).

Sheep Number	Days in gestation	Day 0		Day I		Day II		Day III		Day IV		Day V		Day VI	
		Na	K	Na	K	Na	K	Na	K	Na	K	Na	K	Na	k
352	55	165.6	5.7	171.8	3.1	144.8	14.1	122.0	36.0	85.4	70.0	83.6	71.2	142.4	13.3
312	60	177.6	5.0	166.4	5.2	146.4	17.4	118.0	33.8	104.0	35.2	25.0	106.4	26.3	111.2
307	72	161.2	4.4	146.8	21.3	118.0	48.8	92.0	64.4	114.4	46.8	96.0	64.8	15.6	94.4
337	78	172.0	4.5	152.8	20.9	127.2	49.6	124.4	63.6	101.6	86.0	109.6	63.6	47.1	125.6
319	82	166.0	5.4	146.4	30.4	132.0	49.6	102.8	48.0	70.6	79.2	70.2	86.4	41.4	89.6
334	88	169.6	7.3	136.8	27.0	107.2	35.2	62.4	95.2	37.5	114.4	29.5	120.0	28.5	102.4
327	94	174.6	4.5	141.6	27.4	114.0	48.0	85.8	88.0	-	-	72.2	72.8	31.7	133.6
308	100	161.6	4.4	108.8	41.0	86.0	80.0	54.0	100.0	72.0	85.6	43.3	69.6	12.3	137.6
339	104	178.4	5.0	130.0	13.1	115.2	35.4	100.4	56.0	82.2	76.8	27.2	128.0	17.3	120.0
325	113	183.2	4.4	131.2	18.5	114.0	48.0	52.6	122.0	40.2	62.8	11.0	148.8	23.0	130.4
316	118	176.8	4.3	131.6	24.6	103.6	54.0	61.2	77.2	55.2	85.6	27.0	92.0	5.6	57.2
356	124	183.2	6.5	160.0	5.4	123.2	31.6	142.4	17.4	41.3	120.0	11.4	97.6	6.8	81.6
320	131	169.6	4.5	147.6	28.0	140.4	27.8	116.4	35.2	60.4	79.2	34.7	112.0	10.5	100.8
324	135	177.6	5.4	123.2	69.2	138.0	30.0	136.0	36.4	151.6	14.6	114.0	31.4	126.4	43.0
309	139	160.0	11.5	155.2	9.7	139.2	31.2	122.4	46.4	-	-	106.4	33.2	-	-
Mean		171.8	5.5	143.3	23.0	123.3	40.0	99.5	61.3	78.2	73.6	57.4	86.5	38.2	95.8

TABLE XIII. Original Data From Each of the Twin Foetuses from the Control and Depleted Ewes.

Sheep Number	Age of foetus	Weight of foetus gm	Foetal muscle Na mEq/100 gm	Foetal muscle K mEq/100 gm	Foetal plasma Na mEq/l	Foetal plasma K mEq/l	Amniotic fluid Na mEq/l	Amniotic fluid K mEq/l	Allantoic fluid Na mEq/l	Allantoic fluid K mEq/l	Amniotic Fluid Volume ml	Allantoic Fluid Volume ml
					CONTROL							
306	124	3370	5.3	6.6	147.6	4.8	65.0	4.3	50.2	56.4	710	570
		3387	6.1	7.0	148.8	4.7	119.6	9.8	9.9	73.6	680	500
					DEPLETED							
352	55	37	6.2	4.3	-	-	130.0	10.8	34.4	1.2	138	75
		34	9.0	4.2	-	-	130.4	10.2	99.6	5.1	185	30
337	78	288	9.6	4.4	148.4	6.1	48.0	3.3	101.2	14.1	585	200
		283	9.9	4.3	147.6	6.9	140.4	8.2	107.1	7.4	555	300
339	104	456	7.9	5.1	146.8	4.2	126.4	8.6	32.8	4.0	825	205
		488	8.4	4.8	143.6	4.4	125.6	6.4	56.6	15.7	700	235
325	113	1940	5.6	5.9	135.2	4.2	75.2	4.8	53.4	10.1	500	1025
		1775	6.0	5.5	134.0	4.2	85.8	8.1	49.6	24.4	725	410