rats, however, do live a more independent life than the younger rats, which are continually tended by their mother. It is of interest to note the small amount of activity present in the thyroid.

Despite these complicating factors, however, the middle parts of the curves (Fig. 2, 5–15 days) are exponential and, we believe, represent a constant rate of breakdown of the absorbed protein over this time. Little is known of the behaviour of normal, or denatured or heterologous proteins in rats of this age, and the degradation of denatured protein may normally proceed at a rate quite different from that in the adult rat. Nevertheless, we believe that the cumulative evidence of the demonstration of immunologically active antibodies absorbed from the gut (Halliday, 1955; Halliday & Brambell, 1956), together with the demonstration in the serum of ^{131}I -labelled protein which moves electrophoretically in the position of γ -globulin (Bangham & Terry, 1957), suggests that what we have been observing in these animals is the catabolism of this absorbed antibody γ -globulin. This γ -globulin, which has been absorbed selectively by the gut of these suckling rats, has then preserved its immunological, electrophoretic and metabolic properties as a protein.

SUMMARY

1. The survival of homologous and heterologous whole adult ^{131}I -labelled serum proteins which have

been absorbed from the gut or injected intraperitoneally in rats 7, 14 and 21 days' old has been studied by measuring the total body radioactivity with a ring counter.

2. The survival time of the homologous protein absorbed (60–90 % γ -globulin) in 7- and 14-day-old rats is approximately the same as that of γ -globulin injected into adult rats.

3. The survival time of heterologous (monkey) serum protein absorbed in 7- and 14-day-old rats is at least as long as or longer than that of homologous serum protein absorbed.

4. It is inferred from these results that some of the γ -globulin is not denatured during its absorption from the gut into the blood stream.

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Studies on the Portal Blood of Sheep

1. ABSORPTION OF AMMONIA FROM THE RUMEN OF THE SHEEP

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During recent years there has been considerable interest in the nitrogen metabolism of ruminants, and in the role of rumen micro-organisms in protein digestion. It has become increasingly evident during investigations on various aspects of protein breakdown and synthesis that the microbial activities in the rumen are not always advantageous to the host animal. In particular, the breakdown of food protein to ammonia in the rumen may lead to an appreciable loss of nitrogen to the animal. McDonald (1948*a*) focused attention on this problem when he showed the importance of ammonia production in protein degradation and the ready absorption of ammonia from the rumen of the anaesthetized sheep. Chalmers, Cuthbertson & Synge (1954) have confirmed this finding, again on anaesthetized sheep. However, little attention has been given to

the quantitative relationship between the ammonia concentration in the rumen and in portal blood or to the measurement of the loss of dietary nitrogen in the form of ammonia.

In the experiments to be described it was shown that there was a correlation between rumen and portal-blood ammonia over a wide range of rumen-ammonia concentrations. Leakage of ammonia through the liver into the systemic circulation occurred at relatively low portal concentrations. At higher rumen-ammonia levels it was possible to correlate rumen-, portal- and peripheral-blood ammonia and to relate the levels to the onset and development of toxic symptoms.

A preliminary account of some of this work has been published (Annison, Hill & Lewis, 1955).

EXPERIMENTAL

Animals and surgical techniques

The main experiments were carried out on five adult Clun Forest sheep and additional observations were made on five other sheep. Each animal was stall-fed for at least 2 months before any experiment. During this period an exteriorized loop of the carotid artery and a rumen fistula were prepared in each animal. At a subsequent operation a wide-bore polythene catheter (3 mm. external diam., 1.5 mm. internal diam.) was introduced into the portal vein through the wall of the portal vein itself, or through the substance of the caudate lobe of the liver and into the branch of the portal vein supplying that lobe, or through the main intestinal trunk of the anterior mesenteric vein.

Irrespective of the method of insertion the catheter was always anchored with its tip resting at the porta hepatis and with its free end, after it had passed through the body wall, sutured to the skin. When not in use the catheter was filled with heparin-saline (1000 units/ml. of 0.9% NaCl) and was washed through with heparin-saline at frequent intervals. This method of obtaining portal blood, a full description of which is to be published elsewhere, allows large samples (20–30 ml.) of blood to be withdrawn rapidly and at frequent intervals. This is of importance when analyses such as the estimation of the individual volatile fatty acids of portal blood are required. The disadvantage of the method is that blockage of the catheter usually occurs after 1–3 weeks.

Chemical methods

Blood ammonia. This was estimated by a modification of the method of Conway & Cooke (1939). Within 20 sec. of the withdrawal of the blood 1 ml. volumes were delivered into the Conway vessels directly from 5 ml. syringes. The delivery limits were from points at which one side of the groove holding the plunger spring were adjacent to one edge of the syringe-barrel calibration marks. Syringes were discarded if the mean volume delivered was above 3% from the theoretical volume: the quantities from any one syringe were consistent within $\pm 2\%$. The corrections of Conway (1950) were applied for the ammonia not released in the time allowed for diffusion (20 min.), for the rate of diffusion from blood as compared with water and for the volatile alkaline material liberated from blood by the action of alkali. The correction values were obtained with human and rabbit blood; on repeating the procedures with sheep blood no significant differences were found. With this procedure and these corrections the concentration of ammonia found in the arterial blood of sheep was always less than 0.1 m-mole/l. and usually less than 0.05 m-mole/l. under normal feeding conditions. These findings and also recovery values of 95–105% for ammonia added to blood indicate that the method gives satisfactory results. It is estimated that when the ammonia concentration is above 0.2 m-mole/l. the method is accurate to $\pm 6-8\%$, but larger errors may occur at lower ammonia concentrations.

Blood urea. This was estimated by the Conway method with the urease-phosphate preparation from jack-bean meal (Arlington Chemical Co., New York). The technique described by Conway & O'Malley (1942) was used: the urease was allowed to act for 20 min. and ammonia released with potassium metaborate as the alkaline reagent. Corrections were applied for ammonia from other sources

and for that which did not diffuse out in 40 min.: the errors probably do not exceed $\pm 3-5\%$.

Ammonia in rumen contents. This was estimated by a modification of the Conway (1950) method, described by McDonald (1948*b*).

RESULTS

A number of experiments were carried out on sheep which received diets that gave rise to varying amounts of ammonia during fermentation in the rumen. Each animal was deprived of food for a short period (18–24 hr.) before an experiment started, to allow the rumen-ammonia concentration to attain a steady but slowly decreasing level. Samples of portal blood, peripheral (arterial or venous) blood and of rumen contents were taken, and the sheep was then fed with its usual ration. On some occasions when the sheep ate slowly the ration was

Fig. 1. Portal-blood ammonia with high (A), medium (B) and low (C) rumen-ammonia concentrations. ●, Portal-blood ammonia; ○, rumen-contents ammonia; △, blood urea (carotid artery in A, jugular vein in B, portal vein in C); □, peripheral-blood ammonia (carotid artery in A, jugular vein in B and C). Expt. A: sheep was fed at 0.5–1.5 hr. with 300 g. of hay and 40 g. of casein; at 1.5 hr. 75 g. of casein hydrolysate in 300 ml. of water were placed in the rumen. Expt. B: sheep was fed at 1–3 hr. with 500 g. of hay and 70 g. of casein. Expt. C: sheep was fed at 0–1.5 hr. and consumed 150 g. of straw, 25 g. of sucrose, 40 g. of starch, 5 g. of molasses and 10 g. of salt mixture. Also at 1.5 hr., 200 g. of starch in 300 ml. of water was placed in the rumen.

supplemented by adding casein hydrolysate to the rumen through the fistula. Estimations of the ammonia and urea concentrations of portal and peripheral blood and of ammonia in rumen contents were made in each experiment.

Fig. 1 illustrates three such experiments in which the sheep received diets leading to high, medium and low rumen-ammonia levels. In Expt. 1A a casein supplement was given, in Expt. 1B a smaller amount of casein was fed and in Expt. 1C the sheep was fed on a special diet (McDonald, unpublished; see Annison, 1956) containing appreciable quantities of readily fermentable carbohydrate which reduced the rumen-ammonia concentration (McDonald, 1952; Chalmers & Synge, 1954). In each of these experiments changes in rumen-ammonia concentration were paralleled by changes in portal-ammonia concentration, although the actual ammonia concentrations in the rumen and in the portal blood differed widely. In no experiment was there any significant change in the ammonia content of the arterial blood. There was no indication of any significant differences between the urea concentration in portal and peripheral blood. There did, however, seem to be some correlation between the rumen-ammonia level and the blood-urea concentration. Thus in Expt. 1A, where the rumen-ammonia level was high, the blood-urea concentration increased gradually towards the end of the experiment, whereas in Expt. 1C, where the rumen ammonia was low, no such increase was observed. Similar experiments at different levels of rumen-ammonia concentration followed essentially the same pattern. A more detailed investigation of the factors controlling blood-urea concentration is to be published elsewhere (Lewis, 1957).

During the course of these experiments where widely varying concentrations of rumen ammonia were produced, many simultaneous samples of peripheral venous and arterial blood were examined for their ammonia and urea content. On some occasions significant differences in the levels were observed but no regular pattern emerged.

On a few occasions the portal-ammonia curve did not follow that of the rumen quite so closely as is shown in Fig. 1. Thus in the experiment illustrated in Fig. 2 the rumen-ammonia concentration increased rapidly from the start of feeding, attained its peak about 2 hr. later and declined relatively slowly over the next 6 hr. The portal-blood ammonia concentration, on the other hand, showed a distinct lag period of about 2 hr. before any increase in ammonia content occurred. A clear lag period was observed also in one other experiment, and in a third experiment a slight lag occurred.

No significant increase in the ammonia concentration of peripheral blood was detected in any of the above experiments, although relatively high

(60 m-moles/l.) rumen-ammonia levels were obtained on a number of occasions. Since there is some evidence that in sheep and cattle ammonia can pass beyond the liver and enter the systemic circulation (Dinning, Briggs, Gallup, Orr & Butler, 1948; Head & Rook, 1955; Repp, Hale, Cheng & Burroughs, 1955), an attempt was made to ascertain whether or not there was an hepatic-ammonia threshold in the sheep. The results are shown in Fig. 3 of an experiment in which, after a short control period, the ammonia content of the rumen was increased stepwise by the repeated addition of an ammonium acetate solution through the rumen fistula. Samples

Fig. 2. Lag period in increase in portal-vein ammonia concentration. ●, Portal-blood ammonia; ○, rumen-contents ammonia; □, jugular-vein ammonia; △, jugular-vein urea. Sheep was fed at 0.75–2.0 hr. and ate 300 g. of straw, 100 g. of sucrose, 70 g. of starch, 100 g. of casein hydrolysate, 10 g. of molasses and 15 g. of salt mixture. No supplement was given.

Fig. 3. Portal- and peripheral-blood ammonia following increase in ammonia concentration in the rumen. ●, Portal-blood ammonia; ○, rumen-contents ammonia; □, carotid-artery ammonia; △, carotid-artery urea. Sheep was not fed. Wt. of sheep, 32 kg. Ammonium acetate solution (M) was placed in the rumen: 100 m-moles at 1, 2 and 3 hr. and 50 m-moles at 4.5 and 5.5 hr.

of portal and arterial blood were removed at frequent intervals. As was expected from the results of the earlier experiments, the ammonia concentration of the portal blood increased with that of the rumen contents, although at a much lower concentration. This agreement was maintained even at the highest concentrations of rumen ammonia attained (80 m-moles/l.). No significant change in the arterial-ammonia concentration occurred until the portal blood contained about 0.8 m-mole/l. Above this level the arterial-ammonia concentration increased at almost the same rate as the concentration in portal blood. At about the sixth hour of the experiment the sheep became somnolent, with slow and stertorous breathing. These symptoms were most marked when the arterial-ammonia concentration reached 0.4–0.5 m-mole/l. and gradually passed off as the blood-ammonia content diminished.

Several other experiments were carried out in which the ammonia concentration in peripheral blood was determined after adding repeated doses of ammonium acetate to the rumen. As the rumen concentration reached 60–100 m-moles/l. a slow leakage of ammonia occurred into the peripheral venous blood, and as the blood concentration exceeded 0.6–0.9 m-mole/l. toxic symptoms developed. These resembled those described previously but became progressively more severe as higher blood-ammonia levels were attained, culminating in generalized tetany and death. The toxic effects observed after the administration of ammonium salts are being investigated in more detail, with particular attention to the toxicity of the NH_4^+ ion and to disturbances in acid–base equilibrium.

Fig. 4. Portal-blood ammonia after administration of casein hydrolysate into duodenal fistula. ●, Portal-blood ammonia; ○, rumen-contents ammonia; □, carotid-artery ammonia; △, carotid-artery urea. Sheep was not fed for 20 hr. before experiment, and at 0.75 hr. 70 g. of casein hydrolysate in 150 ml. of water was placed in the duodenum.

Since absorption of ammonia from the small intestine may be of significance under certain conditions, the results of an experiment in which a partial casein hydrolysate (Allen and Hanbury Ltd., Bethnal Green, London: N, 12.7%; free α -amino N, 1.2%) was introduced directly into the duodenum are illustrated in Fig. 4. Approximately 20% of the total nitrogen of this hydrolysate was present as ammonia. Within 30 min. of introducing the casein hydrolysate there was a dramatic increase in the portal-ammonia concentration followed by a rapid decrease during the following 30 min. Thereafter, the portal ammonia remained relatively constant at a somewhat higher level than was found before dosing.

DISCUSSION

These experiments show that in the conscious sheep ammonia is absorbed from the rumen into the portal system, and so confirm the original observations of McDonald (1948*a*) which were made on anaesthetized sheep. McDonald's data were derived from estimations made on blood obtained from one of the rumen veins, and hence the ammonia concentration was greater than that observed in the present experiments. Nevertheless, despite the dilution of rumen venous blood with the remainder of the splanchnic drainage, quite appreciable values for portal-ammonia concentration were found. In the early stages of this work it was thought that ammonia production from sources other than the rumen might detract from the value of portal-blood analyses, but in fact extra ruminal-ammonia production under the conditions of the experiment appeared to be small and constant.

A more serious difficulty associated with the collection of blood samples from the portal vein was related to the possible occurrence of laminar or streamline flow. This has been shown to occur in the dog (Hahn, Donald & Grier, 1945), rat (Himsworth, 1947) and rabbit (Helps & McDonald, 1954), and Garner & Singleton (1953) on the basis of marker experiments with radioactive chromic phosphate found that streamlining, although variable, occurred in the portal vein of the sheep and goat. Schambye (1956*a, b*), on the other hand, concluded from acute experiments in which blood labelled with the plasma dye T. 1824 was infused into the portal vein, and from *in vivo* experiments in which ^{32}P -labelled blood was injected into the splanchnic circulation, that in the sheep there was considerable mixing of the portal blood before it reached the liver. Anatomically, the arrangement of the various vessels draining into the portal vein is such that appreciable mixing might be expected to occur. Nevertheless, it is possible that the results illustrated in Fig. 2 were due to incomplete mixing. However, the fact that on most occasions changes in the rumen were

reflected so closely and rapidly by changes in the portal blood indicated that for most of the time complete mixing occurred.

The close correlation between the changes in ammonia concentration in the rumen and in the portal blood indicated that rapid transference of ammonia occurred through the rumen epithelium into the venous blood draining the rumen. There was no evidence for any mechanism regulating absorption of ammonia from the rumen into the portal blood and hence it seemed most probable that ammonia transference was effected by simple diffusion through the rumen epithelium.

The observation that ammonia was absent from the peripheral blood of animals receiving diets giving rise to appreciable amounts of ammonia indicated the efficiency of the liver in removing ammonia. Since, however, there is evidence that ammonia can enter the general circulation in ruminants (Dinning *et al.* 1948; Head & Rook, 1955; Repp *et al.* 1955) it would appear that the capacity of the liver for converting ammonia into urea can be exceeded when the rumen-ammonia concentration is high. That this was so was confirmed by gradually increasing the ammonia content of the rumen, when a gradual rise occurred in the arterial-ammonia level. The rumen- and portal-ammonia concentrations were probably not critical, since leakage of ammonia through the liver occurred over a range of rumen-ammonia levels and gradually increased as the rumen concentration increased. What appeared to be more significant was the ammonia concentration in the arterial blood at which signs of ammonia poisoning occurred. This concentration was around 0.4 m-mole/l. in the experiment carried out on the sheep with a portal-vein catheter, whereas in the experiments with sheep fitted only with rumen fistulae the concentration was around 0.6–0.9 m-mole/l. This apparent increased susceptibility to ammonia may have been due to the additional surgical trauma involved in catheterizing the portal vein.

During the different experiments the rumen-ammonia concentration at which ammonia appeared in peripheral blood was not constant. Therefore the results of the experiments described above, and of some other comparable experiments, are given in Fig. 5 in the form of a curve in which the concentration of ammonia in rumen contents is plotted against that in peripheral blood. The curve has been drawn with moving averages for units of 10 on the abscissa. It is apparent that ammonia appears in peripheral blood when the rumen concentration exceeds 55–60 m-moles/l. However, Annison, Chalmers, Marshall & Syngé (1954) found rumen-ammonia levels as high as 85 m-moles/l. in sheep receiving diets containing protein supplements, and no toxic effects were observed. On the other hand,

Head & Rook (1955) found a significant ammonia concentration (0.25 m-mole/l.) in the peripheral venous blood of the cow when the rumen ammonia was as low as 30–40 m-moles/l.; signs of ammonia poisoning were not reported. These authors further suggest that under conditions of high rumen-ammonia production ammonia may pass in quantity from the rumen to the small intestine. As part of an investigation into the absorption of materials from the small intestine of the sheep the experiment illustrated in Fig. 4 was carried out, and it is included to show that ammonia absorption can occur from the small intestine and is, in fact, extremely rapid.

Although the experiments of McDonald (1948*a*) and Chalmers *et al.* (1954) have suggested that ammonia production in the rumen may lead to appreciable loss of nitrogen, it has not yet been possible to obtain any quantitative information. This could be investigated by the determination at frequent intervals of blood flow and ammonia concentration in the portal vein, coupled with the assessment of nitrogen excretion. It is, however, of interest at this stage to attempt some approximate calculation of nitrogen loss on the figures which are now available. Schambye (1956*b*) has reported flows of around 37 ml./min./kg. of body wt. in the conscious sheep. In a 40 kg. sheep receiving 800 g. of hay plus 50 g. of casein daily the average rumen ammonia may be estimated at 40 m-moles/l., with a portal-ammonia concentration of around 0.65 mg./100 ml. Assuming an average flow of 1500 ml./min. in the portal vein, then the actual amount of ammonia nitrogen carried to the liver over 24 hr. is in the region of 14 g. Even if a considerable

Fig. 5. Correlation of ammonia concentration in rumen contents and peripheral blood of sheep. $\times$, Moving average values over units of 10 (0–120) or 20 (120–200) m-moles of ammonia/l. of rumen contents; $\bullet$, actual observed points. Results were collected from experiments reported and some other comparable ones: thus conditions of feeding and time were varied.

proportion of this nitrogen is returned to the rumen in the form of salivary urea, the nitrogen loss to the animal must represent an appreciable proportion of the dietary intake. This approximate calculation emphasizes the need for more accurate estimations of nitrogen loss in animals receiving different diets and particularly in animals maintained under normal conditions of husbandry. Attempts are now being made to obtain this information.

SUMMARY

1. A technique is described for taking repeated samples of portal blood in the sheep.

2. Changes in the rumen-ammonia concentration of sheep fed on various diets were paralleled by changes in portal-blood ammonia concentration.

3. At the maximum rumen-ammonia level attained as a result of feeding (60 m-moles/l.), an increase in ammonia concentration was not detected in peripheral blood.

4. When this concentration was exceeded by adding ammonium acetate solution to the rumen, ammonia was present in peripheral blood.

5. As the concentration of ammonia in peripheral blood exceeded 0.6–0.9 m-mole/l. toxic symptoms developed.

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Studies on the Portal Blood of Sheep

2. ABSORPTION OF VOLATILE FATTY ACIDS FROM THE RUMEN OF THE SHEEP

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The nutritional significance of the volatile fatty acids (VFA) acetic, propionic and butyric, which are produced during ruminal digestion, is well recognized, and much information is available on the relative quantities of individual acids which arise under different feeding conditions (cf. Phillipson & Cuthbertson, 1956). Several groups of workers have studied the rates of absorption of these acids from the sheep rumen and the conflicting conclusions which have emerged no doubt reflect the different conditions under which the observations were made (Gray, 1947, 1948; Kiddle, Marshall & Phillipson, 1951; Masson & Phillipson, 1951; Johnson, 1951). Earlier workers studied the absorption of VFA from the rumen of anaesthetized

sheep, but observations on conscious sheep were made by Pfander & Phillipson (1953), although as in the previous investigations the rate of disappearance of VFA added to the rumen was used as a measure of their absorption.

A more direct method of assessing the amounts of VFA and other metabolites which are absorbed from the rumen and other parts of the alimentary tract lies either in the analysis of the blood draining individual organs or in the analysis of portal blood. The study of rumen absorption by the examination of the blood draining that viscus has not attracted a great deal of attention since the now classical work of Barcroft, McAnally & Phillipson (1944). This approach has, however, been adopted by