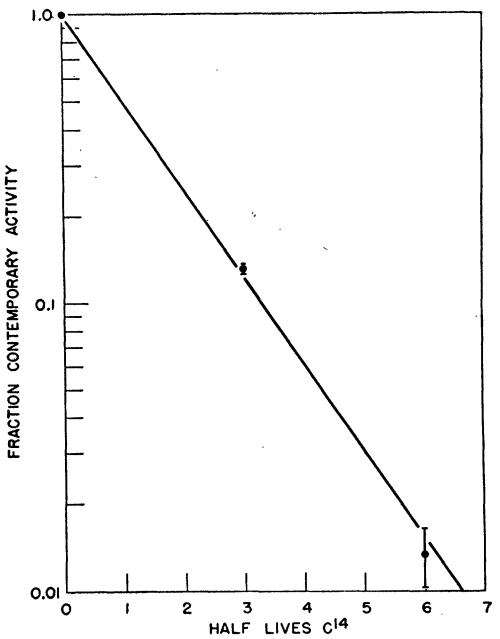

FIG. 3. Count rate vs. "age" for a series of synthetic samples. The line is drawn through the contemporary point with the theoretical slope.

alcohol, and the others were mixtures of 1/8 and 1/64 parts living alcohol with 7/8 and 63/64 parts dead alcohol, respectively. These samples simulate "ages" of 3 and 6 half-lives of C^{14}, respectively. The results are shown in Fig. 3, where the errors shown are the statistical counting errors (standard deviation) alone. The straight line represents the decay of C^{14}. It is seen that the samples agree within the statistical error. The counting data are internally consistent in the same way.

The value of the contemporary assay of natural C^{14} obtained from these data is 15.2 dpm/g carbon. This is in excellent agreement with previous data (*1*), but since our figure is based on an Oak Ridge standard of uncertain accuracy, its value is doubtful.

This method allows us to measure natural C^{14} to a precision of 0.46% in 48 hr, compared to 1.6% for the screen-wall counter technic. Six half-lives are well within reach, as the curve shows. A method for converting samples to aliphatic hydrocarbon is being worked out, so that solutions of good efficiency can be prepared that are 80% sample. This will allow a further extension of two half-lives. The cell size of 100 ml represents an arbitrary choice, and we believe that cells up to 1 liter or more in size are feasible with present equipment. Finally, the experiments of Cowan *et al.* (*8*) indicate that there is no natural limit to sample size, if sufficient starting material is available. We expect that it will be practical to extend the method to the point where its assumptions break down.

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Preliminary Investigations on the Role of Alfalfa Saponin in Ruminant Bloat

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Saponin has been suggested by several investigators (*1–5*) as being a contributing factor in the pathogenesis of ruminant bloat, especially "frothy" bloat. However, its exact role has been open to speculation for a number of years. Jacobson (*6*) isolated a compound from alfalfa that he regarded as a saponin as early as 1919. Boas and Steude (*7*), Jaretsky (*8*), Jaretsky and Lindner (*9*), Henrici (*5*), and Walter, Van Atta, Thompson, and Maclay (*10*) have now definitely established that at least 2 different saponins can be found in alfalfa. The only previous experiment described in the literature to ascertain the effect of feeding ruminants saponin actually derived from alfalfa was performed by Jacobson (*6*). Jacobson fed a sheep 19 g of his saponin preparation and reported that the animal suffered no ill effect. However, serious doubts have been raised as to the identity of Jacobson's product as a saponin because it contained combined nitrogen and did not hemolyze blood (*7, 8*).

The recovery of several pounds of a composite alfalfa saponin was undertaken at the Western Regional Research Laboratory to serve as starting material for isolation of the individual saponins and to provide a sufficient quantity for testing its activity as a factor in ruminant bloat.

This paper briefly describes the method used in the recovery of the alfalfa saponin and the results obtained with it in animal tests. The latter were carried out in the Animal and Poultry Husbandry Research Unit at the Agricultural Research Center.

Recovery of Alfalfa Saponins. The procedure for preparation of the composite alfalfa was as follows: Dehydrated alfalfa was extracted exhaustively with boiling water and the aqueous solution concentrated to about 50% solids *in vacuo*. Alcohol (95%) was added to yield an 80% alcohol solution which precipitated protein, salts, etc. The alcohol solution was decanted, evaporated *in vacuo* to 50% solids to remove alcohol, and the residue boiled with powdered cholesterol. The cholesterol-saponin addition product was filtered off,

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washed with water, dried, and dissolved in pyridine. Saponin was precipitated by addition of anhydrous ethyl ether. The precipitate was filtered off, washed with ether, and dried. Yield was about 0.5% of the dry weight of alfalfa.

Animal Tests. Five yearling ewes, two goats, and one heifer were used in the tests, following preliminary testing for susceptibility to bloating. In this preliminary treatment the animals were pastured on alfalfa or ladino clover for several days and then drenched with ladino clover or alfalfa juice. None of the animals displayed any signs of bloating from natural grazing, but all bloated when drenched with clover or alfalfa juice. This procedure was considered to be necessary, since bloating under natural grazing conditions has been almost nonexistent at the Agricultural Research Center for several years.

The classification of bloat obtained during the tests was based on the following factors: (1) the amount of distention of the rumen, (2) the tightness of the distention, and (3) the discomfort of the animal. A rating of severe has been reserved for a case resulting in death or in which the animal must be treated immediately to prevent death.

In the 10 different tests in which alfalfa saponin was administered to ruminants, definite distention of the rumen was obtained in 8 cases. The distention obtained in these cases was rated from light moderate to moderate bloat when 15 to 25 g of saponin was administered to 5 sheep and 1 goat, moderate to severe when 55 g of saponin was given to another sheep, and light moderate when 75 g was given to a heifer. During the tests the saponin was dissolved in 1 pt to 1 qt of water and administered to the animals by using a stomach tube. In general, the height of the distention of the rumen occurred in 30 to 45 min with the alfalfa saponin as compared to 10 to 15 min when using alfalfa or ladino clover juice drenches. Only a very slight distention was produced when 15 g of alfalfa saponin was given to ewe No. 44 while grazing on a grass pasture. No distention of the rumen, however, has been produced in a number of attempts where sheep, grazing on a grass pasture, were drenched with ladino clover juice or alfalfa juice. When ewe No. 44 was grazing on ladino pasture, 25 g of the alfalfa saponin produced a distinct distention of the rumen. No reaction was observed when 15 g of alfalfa saponin was given to a mature goat. However, this goat required twice as much ladino clover juice to produce a distinct distention of the rumen as was required by the sheep used.

In all cases, the distention appeared to be due to gas retention rather than froth, since the passage of a stomach tube into the rumen permitted an immediate release of gas and reduction of distention.

No detectable distention of the rumen was produced by giving two to three times the amount of water used in the above tests. In subsequent tests, all the sheep were each given 50 g of a commercial 50% saponin solution labeled by the manufacturer as nontoxic and 25 g of another commercial saponin preparation labeled as being toxic. Both of these products were isolated from the yucca plant. These materials were administered in either 1 pt or 1 qt of water and produced no detectable reactions. The commercial saponin preparations appeared to have as strong foam-producing qualities as the alfalfa saponin. Twenty-five grams of a household detergent in 1 pt to 1 qt of water also produced no reaction with the above animals. The heifer used in the tests has been given this detergent in water, in combination with sugar, and with sugar and aeration of the rumen with oxygen, without reaction in any of the tests.

Investigations on the mode of action of alfalfa saponin in ruminal bloat are being continued.

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A Study of the Relationship between Asymmetric Acetylcholinesterase Activities in Rabbit Brain and Three Behavioral Patterns

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Involuntary behavior such as the forced circling preceding certain types of epileptic attacks is frequently associated with a lesion in a specific area of the cerebral cortex. Such compulsive turning also has been produced experimentally by extirpation of a portion of the frontal cortex in monkeys (*1*). Mettler (*2*) has shown that bilateral frontal lobectomy plus unilateral caudate ablation in cats may also result in forced circling. The animal may progress in a straight line, but more usually it circles and occasionally will spin on its hind legs. In most cases the circling is toward the side of the caudate lesion, but it may be in the opposite direction. It should be pointed out that the mechanism of forced circling probably involves additional structures in the central nervous system (*3, 4*).

It has been previously demonstrated that a biochemical lesion of the brain can be produced by the intracarotid injection of diisopropyl fluorophosphate (DFP) which under this condition inhibits the activ-

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