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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Manuscript received November 24, 1953.

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-

[1] The authors acknowledge the valuable technical assistance of Madeline Griffin and Shirley Moak.

TABLE 1. Acetylcholinesterase activities expressed as percent of normal in rabbits after intracarotid injection of DFP.

Tissue	Data Expressed as Percent of Control*		
	"Left-ers"	"Right-ers"	"Neu-trals"
Right cortex	29	27	86
Left cortex	90	54	88
Right caudate nucleus	3	6	82
Left caudate nucleus	83	84	98

* Nine rabbits were used for the control series and 6 rabbits for each of the other categories. "Lefters" turned away from the injected right side, "righters" turned toward the injected side, and "neutrals" did not exhibit compulsory behavior.

ity of acetylcholinesterase (AChE) in the parts of the central nervous system irrigated by branches of the common carotid artery (*5, 6*). This lesion evokes compulsory turning of the animal in a direction away from the injected side (*6, 7*). Although forced turning away from the injected right side ("lefters") is the pattern usually observed, it was noted that sometimes the direction of the turning is reversed, i.e., toward the injected side ("righters"), and in some cases the animal does not exhibit any compulsory behavioral pattern ("neutrals").

In our experiments 0.1 mg/kg of DFP was injected into the right common carotid artery of rabbits weighing approximately 2 kg, in order to study the enzymatic changes that were associated with the behavioral responses. The AChE activity of the frontal cortex and caudate nucleus on both sides of the brain was measured in animals exhibiting each of the three behavioral patterns. A series of control animals was obtained by substituting water for the DFP in the carotid injections.

After a 20-min period in which a single behavioral response became well established, the animal was sacrificed by an injection of air into the marginal ear vein. The tissue to be analyzed was then removed and the AChE activity was measured by a continuous titrimetric method. The enzyme activity was calculated and expressed as mg of ACh hydrolyzed per mg of wet tissue per min. A more detailed study and description of the method will be reported elsewhere.

The AChE activities are expressed as percent of normal in Table 1. The data reveal a general decrease of AChE activity in the tissues studied following the intracarotid injection of DFP. However, in "lefters" and "righters" this decrease was very much greater on the right side of the brain than on the left. This large difference between the two sides, or asymmetry of enzyme activity in the cortex and caudate nucleus, was noted in every rabbit that exhibited the circus movements irrespective of the direction. In "righters" the AChE activity for the left cortex is relatively low when compared to the "neutrals." However, the asymmetry of AChE activity between the left and right cortices is still present because the right side has suffered a far greater decrease than has the left. That this asymmetry of AChE is associated with forced circling is further emphasized by the fact that it is absent in the "neutrals."

We have thus been able, by the production of an asymmetric biochemical lesion, to duplicate behavioral patterns previously obtained by extirpation or by electrical stimulation of specific cerebral areas. We now find that a characteristic AChE pattern in the cortex and caudate nucleus is associated with each behavioral response. These results further support the concept that cortical and subcortical structures are involved in forced turning (*2*).

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Manuscript received October 26, 1953.

Rotating Sector Method Applied to Reactions Induced by Co^{60} Gamma Rays

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Intermittent illumination has been extensively used in photochemistry but its application in radiation chemistry has been much more limited (*1*). This has been partly due to technical difficulties associated with the penetrating nature of the radiations used and partly to a lack of sufficiently strong sources of radiation. Hart and Matheson (*2*) obtained intermittent illumination of their samples by placing them on the circumference of a wheel rotated in front of an orifice in a lead shield behind which there was an 80-curie cobalt source. In the present work a rotating sector was constructed which could be placed between a 1000-curie cobalt source and the samples to be irradiated, thus allowing the samples to remain stationary during irradiation. The sector was a solid steel cylinder, 6 in. in diameter, 1 ft long, with two 60° sectors cut out on opposite sides. It was connected through a series of pulleys to a variable speed motor (see Fig. 1).

Two systems have been investigated, one molar aqueous chloral hydrate solution and chloroform saturated with water. These particular systems were chosen

[1] The authors are indebted to the Defence Research Board of Canada for financial assistance, and to the Saskatchewan Cancer Commission for the use of the 1000-curie cobalt unit.