a 100-ml sample of extract was distilled at atmospheric pressure. The distillate was collected in three 30-ml fractions, and the residue brought back to the original volume with redistilled water. Some of the distillates inhibited autoxidation, but, since it was not always possible to detect the inhibitory factor(s) in the distillates, it was felt that volatility of the factor(s) must depend on some unknown condition. Since the pH of the distillates was in the alkaline range (7.5–8.5), additional distillations of snap-bean extract were carried out, using alkaline snap-bean extracts that had been prepared by blending snap beans with solutions of dibasic sodium phosphate instead of water. It was found that as the pH of the snap-bean extract increased, the volatility and stability, as shown by storage studies, of the inhibitory factor(s) apparently increased, though at the high pH values (above pH 8), the extract itself did not show its usual strong inhibition of ascorbic acid autoxidation.

These results strengthen the existing evidence that vegetables contain an inhibitor(s) of ascorbic acid oxidation. Some of the properties of the inhibitor(s) in snap beans are also indicated. Further work is in progress to define more definitely the nature of the inhibitor(s).

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Prolongation of Clotting Time in Dormant Estivating Mammals[1]

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During an investigation of the seasonal changes in blood volumes of ground squirrels of two species (*Citellus columbianus* and *C. parryii ablusis*), it was discovered that a prolongation of the clotting time of the blood normally occurs in these animals when they are in a dormant state.

The blood volumes were determined by Cartland and Koch's (*1*) micromodification of the Keith-Rowntree-Geraghty (*2*) plasma dye dilution method. Because of the relatively small size of the animals and the lack of large superficial blood vessels, sufficient blood could not be taken readily from the ears or tails as is ordinarily done in other experimental animals. Hence heart punctures, using a #23 hypodermic needle, were resorted to. By this method adequate amounts of blood could be secured, and blood volumes satisfactorily determined while the animals were still in an active state. When the animals became dormant either during estivation or hibernation, this method proved unsatisfactory. The primary disadvantage was that the animals usually died shortly after the blood samples were taken. It was revealed upon post-mortem examinations that death was caused by internal hemorrhages, the pericardial cavity being completely filled with blood. When a finer-gauge needle (#26) was substituted, blood samples could be taken without the accompanying pericarditis and resultant death.

It was at first assumed that the internal hemorrhages were due to faulty technique and that death was caused by a mechanical injury to the heart or adjacent blood vessels. However, when samples of the blood of the dormant animals were exposed to the air, they did not clot normally even after an exposure of several days. Hence death was not due to faulty technique but to a hemophilic condition of the blood.

Comparative studies on the clotting time of the blood of both active and dormant ground squirrels were then made. Three regular techniques, capillary tube, Lee and White's, and Howell's, were used. These methods of determining clotting time differ from each other essentially in the diameter of the tubes. The capillary tubes have the narrowest lumen, those used in Howell's technique the widest. As can be seen from Table 1, all produced similar results.

TABLE 1

No. animals tested	No. blood samples	Clotting time (min and sec) Minimum	Maximum	Av
		Capillary tube method		
16 active	30	0′ 33″	12′ 0″	4′ 34″
11 dormant	15	10′ 51″	51′ 45″	20′ 6″
		Lee and White's method		
10 active	10	3′ 41″	29′ 30″	14′ 36″
7 dormant	9	16′ 43″	39′ 0″	26′ 25″
		Howell's method		
5 active	5	4′ 30″	13′ 20″	8′ 12″
5 dormant	5	35′ 23″	68′ 30″	48′ 43″

The term active refers to all animals that were not in a truly dormant state and includes those that were drowsy. Some squirrels remained in the mid-state of drowsiness, neither completely active or completely dormant. The longest periods on clotting times for active squirrels were obtained from these drowsy animals.

In the really active ground squirrels a complete clot occurred and determined the end point of clot formation, whereas in the dormant animals a complete clot never did form. Only a partial clot formed even if the blood was exposed for several days.

The prolongation of blood-clotting time may be considered a remarkable adaptation of estivating

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mammals to the dormant state. During estivation the heart beats very slowly, with a comparable decrease in the rate of blood flow, and hence any mechanism to prevent the formation of a thrombosis during the inactive state would be of considerable survival value to the animal. In the active state a hemophilic condition of the blood would, on the contrary, be a distinct detriment, for any slight injury involving bleeding could lead to death. In estivation, since the animals are buried underground in their nests, injuries are very unlikely.

The various factors involved in this prolongation of clotting time are being studied.

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Reversed Phase Partition Chromatography of Steroids on Silicone-treated Paper[1]

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The conventional procedure of paper partition chromatography, in which the paper holds water as a stationary phase, is not satisfactory when applied to a class of compounds such as the steroids, which have limited water solubility. This difficulty can be circumvented in a number of ways.

One solution would consist of preparing derivatives having greater water solubility. Zaffaroni and co-workers (*1*) prepared the Girard T derivatives of a large number of steroid ketones, and determined their R_F values on filter paper using a water-butanol partition system. Their procedure, although ingenious, does not give satisfactory separations. Other difficulties involve the instability of the Girard T derivatives and also the inconvenience entailed in their preparation.

A second approach would concern itself with a modification of the partition coefficient in favor of the polar, stationary phase, by incorporating a polar phase other than water in the paper support.

An example of this solution can be found in another method developed by Zaffaroni and co-workers (*2*) for the separation of the more water-soluble adrenal cortical steroids on paper. These investigators impregnated filter paper with either formamide or propylene glycol, which served as the stationary, polar phase, and used benzene or toluene, respectively, as the mobile, nonpolar phase.

A third approach, which has been suggested for the chromatography of compounds whose partition coefficients are greatly in favor of the mobile, nonpolar phase, consists of reversing the two phases. In other words, the supporting substance, paper, silica, kieselguhr, or glass, is treated in such a manner as to hold the nonpolar phase stationary. The more polar phase is then employed as the moving one. Within the past few years a variety of procedures has been described for the production of reversed phase systems and for application of the reversed phase technique (*3–7*).

The partition chromatogram is most effective when the compounds to be separated have R_F values in the range 0.3–0.5. We believed that the application of the reversed phase technique to the problem of separating the less polar steroids would aid in the realization of these conditions.

In this report we wish to present a procedure by which we have been able to separate steroid mixtures by application of reversed phase partition chromatography on filter paper. A paper manufactured in Sweden (Munktell 20, 150 G) was found to be most satisfactory. Strips 8 × 48 cm were drawn through a 5% (by vol) solution of Dow Corning Silicone No. 1107 in cyclohexane. These strips were then blotted between sheets of an adsorbent paper to remove excess solution and placed in an oven at 110° C for 1 hr. The paper was rendered hydrophobic by this treatment and readily adsorbed vapors of nonpolar substances such as chloroform. The properties of the treated paper are not changed by washing with organic solvents.

The most satisfactory partition system employed for the separation of the group of steroids used was prepared by mixing 6 vol water, 10 vol absolute ethanol, and 10 vol reagent grade chloroform. The two phases separated in a few minutes but were allowed to stand at room temperature for 1 hr before use, the upper phase being the more polar.

Usually 3 mixtures were analyzed on one paper. The 3 mixtures in chloroform solution were applied at 3 different spots at 2-cm intervals along a line 6 cm from one end of the paper. The mixtures to be analyzed contained 10–25 γ of each component. The paper was then placed in an airtight glass cylinder, the atmosphere of which was kept saturated with vapors of the nonpolar phase. After allowing 1 hr for the incorporation of a stationary phase in the paper by adsorption of the vapors, the chromatogram was developed with the more polar alcohol-water phase in the conventional descending manner. When the moving solvent had run the desired distance (35–40 cm) the paper was removed and dried at 110° C for 10–15 min. It was then sprayed with a freshly prepared mixture of equal volumes of a 2% solution of *m*-dinitrobenzene in absolute ethanol and a 2.5 *N* solution of potassium hydroxide in absolute ethanol. In order to achieve maximum development of color, the paper was placed in a 110° oven for 30–60 sec. The distance traveled by a compound was measured from the origin to the center of the spot.

Because of the sensitivity of the R_F values to small differences in the amount of silicone incorporated in the paper, and also because of the many other factors (*8*) which affect the reproducibility of R_F values, we

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