base balance on the excitability of the central nervous system, convulsions were generally induced by drugs or electric shock. In some cases, anesthetized preparations were used (*5*). The present work and previous studies on audiogenic seizures (*1, 2*) confirm that CO_2 accumulation tends to depress central nervous system excitability and show that this is true in the intact animal.

It has been postulated that the anticonvulsant action of acetazoleamide is due to direct brain carbonic anhydrase inhibition (*6*). There is little doubt that acetazoleamide does inhibit brain carbonic anhydrase, however, in view of increasing evidence suggesting respiratory acidosis resulting from blood carbonic anhydrase inhibition after administration of large doses of acetazoleamide (*3, 7*); it seems premature to ascribe the anticonvulsant effect of acetazoleamide to such a localized action.

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25 August 1955

Use of Glycine in Bovine Semen Extenders Stored at 5° and – 78°C

The survival time of bovine spermatozoa has been increased with the use of a 3-percent glycine extender beyond the time, obtained with use of a 3.2 percent sodium citrate extender when stored at 4°C (*1*). This advantage of glycine over sodium citrate was believed to be due to the low electrolyte content and to the maintenance of the functional integrity of the sperm cells. Evidence has been presented to indicate that amino acids play an important role in the physiology of the sex organs (*2*). Further evidence has been presented to demonstrate that certain amino acids increase the life span of sea urchin spermatozoa, but that they are not used metabolically. It has been found that glycine extends the viability of fowl spermatozoa (*3*).

Gassner and Hopwood (*4*) have reported that the amino acids found in bovine semen, in order of apparent concentration, are glutamic acid, alanine, glycine, serine, and aspartic acid. The concentration of any of the five amino acids is about the same in both plasma and whole semen.

Tyler and Rothschild (*2*) observed that when whole semen was incubated for 3 hours to permit metabolism of fructose by spermatozoa, none of the amino acids were utilized. The purpose of amino acids in semen appears to be other than for metabolic functions. Since they are amphoteric in reaction, they may be a part of the natural buffer system. Glycine was selected as the amino acid to be used in this study because of its natural occurrence in semen and because of the indication of its possible use that was given by other workers (*1*).

Three-percent glycine in double-distilled water is not isotonic to bovine semen. Freezing-point determinations in this laboratory indicate that 2.05-percent glycine in double-distilled water with added antibiotics is isotonic to semen. It was decided to test the 2.05-percent and the 3-percent levels of glycine against 2.94-percent sodium citrate (dihydride), which would serve as a control, since it has been in common use in bovine semen extenders for many years.

The semen was collected from dairy bulls in an artificial vagina. It was transferred immediately to a test tube that was stoppered to prevent contamination. The semen was then divided into three equal portions and extended with the following: (i) an extender made up of 50 percent egg yolk and 50 percent by volume of 2.94-percent sodium citrate (dihydride) solution; (ii) an extender made up of 50 percent egg yolk and 50 percent by volume of 3-percent glycine solution; and (iii) an extender made up of 50 percent egg yolk and 50 percent by volume of 2.05-percent glycine solution. The rate of extension was 1 part bovine semen to 20 parts of extender.

Table 1. Influence of glycine-yolk and citrate-yolk extenders on the motility of bovine semen stored for 7 days at –78°C.

Extender	Ejaculates (No.)	Avg. motility (%)
3% glycine	10	50.8 ± 5.36
2.05% glycine	10	49.7 ± 5.66
2.9% citrate	10	50.7 ± 6.16

The antibiotic levels used were 0.2 g streptomycin and 0.06 g penicillin per 100 ml extender. The extended semen was then cooled in a refrigerator at 5°C for 5 hours. A small sample was then taken for storage at 5°C. The remaining portions were extended by adding at a rate of 1 to 1 a solution consisting of (i) 16 percent glycerol by volume in 2.9-percent sodium citrate (dihydride) solution; (ii) 16 percent glycerol in 3-percent glycine solution; and (iii) 16 percent glycerol in 2.05-percent glycine solution, respectively. The final concentrations of the components of the three extenders were as follows: (i) 67 ml of 2.9-percent sodium citrate (dihydride) solution, 25 ml of egg yolk, and 8 ml of glycerol; (ii) 67 ml of 3-percent glycine solution, 25 ml of egg yolk, and 8 ml of glycerol; (iii) 67 ml of 2.05-percent glycine solution, 25 ml of egg yolk, and 8 ml of glycerol.

The extended semen was then sealed in glass vials and equilibrated for 18 hours at 5°C. It was then frozen in a bath containing acetone and Dry Ice. The rate of freezing was 1°/min from 5° to – 15°C and 3 to 4°/min from – 15° to – 72°C. The samples were transferred to a storage cabinet containing Dry Ice in methanol and stored at – 78°C. The samples were thawed on the seventh day and the percentage of motile spermatozoa was observed. The results of this trial are presented in Table 1. An analysis of variance indicated that the differences observed were not significant. The motility observations on the extended semen stored at 5°C are presented in Table 2. After 4 days of storage, there appeared to be little difference between extenders. After 6 days of storage, the glycine-yolk extenders appeared to give better results than the citrate-yolk extenders. The *p*H observations indicate that glycine does not buffer the extended semen as well as sodium citrate.

Table 2. Influence of glycine-yolk and citrate-yolk extenders on the motility and *p*H of bovine semen stored at 5°C.

Storage (days)	Ejaculates (No.)	Avg. motility (%)			Storage (days)	*p*H of extended semen		
		2.05% glycine	3% glycine	2.9% citrate		2.05% glycine	3% glycine	2.9% citrate
2	10	64.4	65.4	63.4	1	6.45	6.40	6.78
4	10	58.4	59.4	58.7	5	6.32	6.32	6.73
6	10	48.4	45.0	40.0				

We conclude that glycine-yolk extenders are equally as good as citrate-yolk extenders under the conditions of this experiment.

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22 August 1955

Absence of Indoleacetic Acid in the Apple

Indoleacetic acid (IAA) is now generally regarded as an auxin of widespread and perhaps universal occurrence in plants. The evidence for this view is based largely on the occurrence on paper chromatograms of plant extracts of an acidic substance that has an R_f value similar to that of synthetic IAA, which is active in promoting cell extension growth in coleoptile sections and which may give characteristic, although not highly specific, color reactions with Salkowski and Ehrlich reagents.

In the acid fraction of ether extracts of both fresh and dried apple seeds, pollen, fruits, and leaves we have found an auxin with an R_f value in butanol-ammonia of 0.30 to 0.45, which is close to that of IAA (R_f 0.25 to 0.40). This auxin, which seems to be the most characteristic and widely distributed auxin in the apple, has been referred to in a previous publication as *Malus* auxin 2 (*1*). Hancock and Barlow (*2*) also found such an auxin in the acid fractions of extracts of young apple leaves and concluded that it was probably IAA. The failure of this auxin to give the characteristic color reactions of IAA even when, according to its biological activity, it was present in sufficient amount, led us to investigate more critically the question of its identity. On the basis of evidence presented here, we conclude that this auxin is not identical with IAA; neither have we obtained, in the course of these investigations, any evidence that IAA occurs in apple tissue.

The absence of typical IAA color reactions is not the result of the presence on the chromatogram of inhibitors, because authentic IAA cochromatographed with the unknown yielded typical IAA color reactions.

The R_f of biological activity in these chromatograms was usually somewhat greater than that of authentic IAA (Fig. 1). A critical comparison of R_f values was made by eluting material from the center of the active spot and rechromatographing this in butanol-water-ammonia. To an adjacent spot of the same extract, 1 μg of IAA was added; after development, this chromatogram was tested with Salkowski reagent. It would appear from this experiment that *Malus* auxin 2 in this solvent has an R_f value approximately 10 percent higher than that of IAA (Fig. 2). It should be noted that a difference in R_f value of this magnitude would scarcely have been detected by the technique of Hancock and Barlow (*2*), who divided their chromatogram strips into only ten portions preparatory to biological assay.

Other solvents such as distilled water, which is recommended by Sen and Leopold (*3*), give more definite separation of the unknown auxin and IAA. *Malus* auxin 2 was eluted from butanol chromatograms with 90-percent ethanol. The eluate was rechromatographed with water as a developing solvent. The R_f of authentic IAA was 0.95 and that of *Malus* auxin 2 was 0.4 to 0.6 (Fig. 3). The double peak given by the unknown auxin on these water chromatograms is due to an inhibitor with approximately the same R_f value, which causes a depression of coleoptile extension in the center of the auxin spot. In addition to this inhibitor, which shows up as a yellow spot after it is sprayed with Ehrlich's reagent, two further compounds separated out on these chromatograms. One of these, at R_f 0.25 to 0.35, gave a pink Ehrlich reaction, while the other could be detected by its bright blue fluorescence under ultraviolet light; neither appeared to be biologically active. No Ehrlich reaction was obtained in a position corresponding to the unknown auxin.

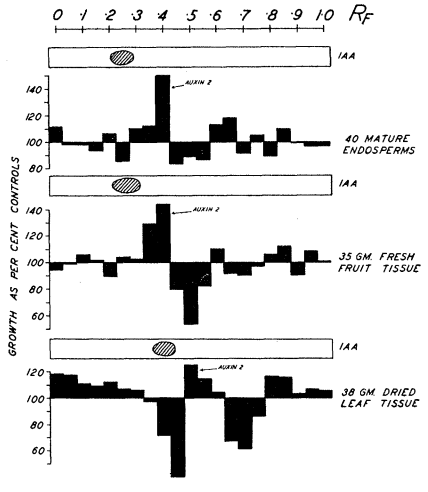

Fig. 1. Chromatograms of the acid fraction of ether extracts of various apple tissues, developed in butanol-ammonia and assayed in the wheat coleoptile section test. The R_f of IAA in each test, as determined by the Ehrlich color reaction, is shown.

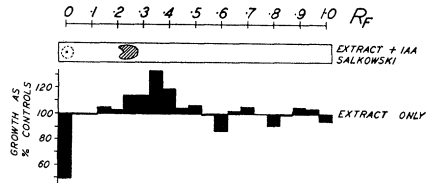

Fig. 2. Auxin 2, eluted from chromatogram and rechromatographed in butanol-amonia, with and without added IAA. Auxin 2 was detected by assay in the wheat coleoptile section test and IAA was detected by Salkowski reaction.

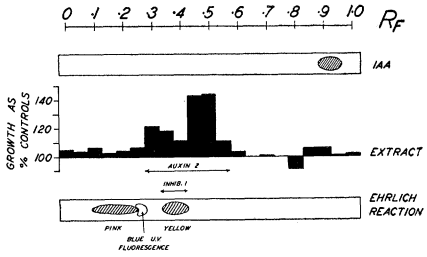

Fig. 3. Auxin 2, eluted from butanol chromatogram of leaf extract and rechromatographed in water, with and without added IAA. Chromatograms tested with Ehrlich's reagent and in the wheat coleoptile section test. A clear separation of IAA and auxin 2 is shown.

Besides its activity in promoting cell elongation, this auxin is active in stimulating root formation on the hypocotyls of *Phaseolus vulgaris* and in delaying the abscission of debladed petioles of *Coleus*, even though, like IAA, it appears to be inactive in the tomato ovary test (*4*). Its failure to give a color reaction with Ehrlich's reagent, even when it is present in amounts equivalent to 0.3 μg of IAA, suggests that it may not even be an indole compound, but this cannot be stated definitely on the basis of the present evidence. The absence of IAA from the apple is particularly interesting in view of its apparent widespread distribution in other plants, including at least one other member of the Rosaceae (*5*).

Malus auxin 2 appears to be the only auxin present in the flesh of the apple; in the seeds and leaves, two other unknown acid auxins occur in addition to at least one neutral auxin (*1*). Teubner (*6*) claims that he has identified the neutral auxin of the apple endosperm as ethyl indoleacetate. However, his identification is based only on a similarity in R_f values in butanol-ammonia and on color reactions, both of which are unreliable when one is dealing with neutral auxins. In view of the apparent absence of IAA, it would seem that Teubner's claim that he has found the ethyl ester should be critically reexamined. His further assumption