Pyridoxal contains groupings that make plausible chelation of the alkali metals, either in aqueous or lipoid phases. Furthermore, the reaction probably produces a neutral molecule that might carry the metal ion through cellular boundaries. Concentration gradients of the metal ion might be established if the molecule undergoes a secondary modification (for example, phosphorylation or displacement of the metal ion by an amino compound) on one side of the phase boundary.

Significantly, the metals that chelate more strongly with pyridoxal (Mg^{++}, Li^{+}, and Na^{+}) are the ones that tend to cause the apotryptophanase-pyridoxal phosphate system to dissociate, whereas K^{+} and Rb^{+} tend to stabilize the holoenzyme (*10*).

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12 September 1955

Electrophoresis of Serum Proteins in a Viscous Film

In the study of proteins by the technique of filter paper electrophoresis, convection or diffusion and adsorption of proteins to the paper with resultant trailing are among the limiting factors in the resolution that can be obtained (*1*). With a protein solution containing 98 percent albumin and 2 percent γ-globulin, the densitometer curves obtained from filter paper electrophoresis in our laboratory indicate that 10 to 15 percent of the albumin is lost in trailing. The work of Brakke (*2*) with graded density columns of sucrose, and of Philpot (*3*) who conducted electrophoresis employing a multilayered solution, suggested the possibility of electrophoresis in a viscous film. We felt that the viscosity and surface forces might be related to the ability of such a film to serve as an anticonvection medium and that the use of a fluid rather than a solid electrophoresis medium would tend to decrease trailing.

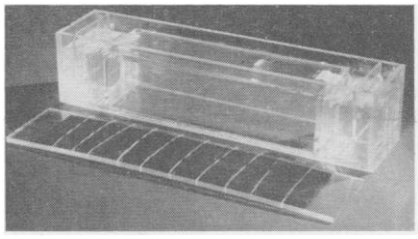

Fig. 1. Plexiglas apparatus set up for film electrophoresis.

An electrophoresis apparatus was constructed of Plexiglas for this purpose; it is shown in Fig. 1. It consists of a longitudinal platform between two electrode vessels containing platinum electrodes and labyrinthine dividers. The experiments were conducted by placing a glass plate 1⅞ by 10 inches on the shelf and a film on the glass plate. After investigating several substances, we used the following method to provide a viscous film of suitable properties. Agar-agar, 0.13 g, was added to 100 ml of barbiturate buffer of *p*H 8.6 and ionic strength 0.05. This was then heated and boiled for 1 minute, until the agar-agar dissolved. Methyl cellulose, 0.028 g, was then added to the solution and thoroughly mixed. The resultant solution is near the gel point, containing slightly less than the amount of agar-agar necessary for gel formation. While the solution was still warm, it was added with a pipette to the glass plate to form a film about 1/16 inch thick.

The film was then connected to the buffer in the electrode vessels by means of filter paper wicks, covered, and voltage applied for 10 minutes, after which more of the solution used to make the film was added if the thickness of the film had decreased. (The surface of the film should be smooth, and it should exhibit interference colors from reflected light. If the concentration of agar-agar is increased above the amount used, there is a decrease in the protein mobility and in the resolution, and the dried film tends to crack and peel off. If a smaller concentration is used, the resolution again appears to decrease.) The serum was then added by means of placing a small piece of filter paper, about 10 mm by 4 mm, which had been soaked in the serum, on the film. The cover of the apparatus, lined with wet blotting paper, was sealed with Saran wrap in order to prevent evaporation. If this precaution is observed, the film is still liquid at the end of the run, and it will run down the glass plate if the glass plate is significantly tilted. It was found that a suitable separation could be obtained by the application of 100 v for a period of 16 hours. The current obtained at 100 volts with this setup is about 3 ma.

When the electrophoresis has proceeded a sufficient length of time, the protein pattern can be obtained by two different procedures. A slightly damp strip of filter paper can be carefully placed over the film and the glass plate inserted into a drying oven at 120°C. As soon as the film is dry, the filter paper can be removed and developed in the usual manner (*4*). An alternative procedure is to dry the film as such, without any filter paper, insert the glass plate in an alcoholic solution of bromphenol blue, wash the film briefly with 1-percent acetic acid, and redry the film by placing the glass plate in the oven again. The glass plate can then be inserted directly into a densitometer, and the optical density curve can be obtained. The protein pattern can be accentuated by placing a blank strip of filter paper between the plate and another glass plate that is blank.

The mobility on the film is approximately the same as it is on filter paper. Albumin stained with bromphenol blue was found to migrate at about 0.65 cm/hr at 100 v. (This includes a velocity component in the opposite direction owing to electroosmosis.) The distance of migration is linear with time.

Figure 2 shows the results obtained

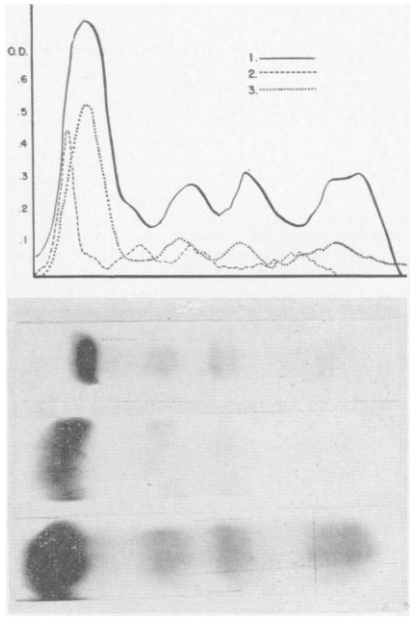

Fig. 2. Electrophoresis strips and corresponding densitometer patterns of the same sample of serum made by three different procedures: curve 3 and top strip show serum run on a film and developed on glass, curve 2 and middle strip show serum run on a film and picked up on paper, curve 1 and bottom strip show serum run on paper by conventional procedure.

with the same sample of serum by three different procedures: (i) curve 3 and top strip show electrophoresis run on a film and developed on the glass; (ii) curve 2 and middle strip show electrophoresis run on a film and developed by picking up the protein pattern on filter paper; (iii) curve 1 and bottom strip show the usual method of filter paper electrophoresis done by the method of Köiw (*4*) and employing 130 v for a period of 15 hours.

Definite conclusions concerning the relative amount of trailing in the top and bottom strips cannot be drawn from Fig. 2, because the amount of serum applied differs in the two instances. The use of the film does appear, however, to provide favorable resolution with relatively little trailing, compared with reports of results obtained with filter paper (*1, 4*). The preparation of the film and setting up of the experiment require approximately 1 hour. It seems probable that the results obtained with films can be improved over those reported here and that the electro-osmosis can be eliminated by the use of suitable film thickeners (*2*). Further experiments are being conducted along these lines.

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14 July 1955

Oxalate Content of Tropical Forage Grasses

It is generally known that the average milk production of the dairy cow in the tropics is far below that of the dairy cow in the temperate zones. Average annual production per cow in India is about 400 pounds (*1*) and in Puerto Rico it is about 2000 pounds (*2*). In the United States, the average annual production per cow is well over 5000 pounds, with sectional averages of more than 8000 pounds (*3*).

The various agencies of Puerto Rico that are concerned with the improvement of dairy herds have shown that, under proper management, the production of dairy herds in Puerto Rico can equal that of similar herds in the United States (*4*). These agencies are very optimistic about the prospects for increasing greatly the milk production of herds in Puerto Rico.

One of the many complex factors involved in proper management is the careful consideration of the quality and chemical composition of the forage grasses that are fed to the herd. During the last few years, improvement in the quality of feedstuffs has been credited with more than 50 per cent of the increase in milk production in New Zealand (*5*). In all countries where highly productive dairy herds have been developed, a comprehensive knowledge of the chemical composition of the forage crops has aided materially in increasing milk production.

Table 1. Oxalate content of tropical forage grasses harvested at 6 to 10 weeks of age

Scientific name	Common names	Oxalic acid (% dry weight)
Andropogon carricosus	Cuban grass	0.25
Axonopus compressus	Carpet grass	0.02
Bouteloua heterostega	Lamilla	0.02
Chloris inflata	Mexican bluegrass	0.43
Cynodon dactylon	Coastal Bermuda	0.16
Cynodon dactylon common	Common Bermuda	0.02
Cynodon plectostachyum	Star grass	0.09
Digitaria decumbens	Pangola	0.89
Eriochloa polystachya	Malojilla	0.22
Melinis minutiflora	Molasses grass	0.41
Panicum maximum var. borinquen	Borinquen	1.10
Panicum maximum var. common	Guinea grass	2.01
Panicum maximum var. gramalote	Guinea grass	1.05
Panicum maximum var. broad-leaf	Broad-leaf guinea grass	2.26
Panicum maximum var. fine-leaf	Fine-leaf guinea grass	1.65
Panicum purpuracens	Pará grass	1.24
Paspalum fasciculatum	Venezuela grass	0.02
Paspalum plicatulum	Sweet grass	0.02
Pennisetum ciliare	Buffel grass	0.83
Pennisetum purpureum var. *merkerii*	Merker grass	2.48
Pennisetum purpureum	Elephant grass	2.57
Sporobulus indicus	Cerrillo	0.22
Sporobulus virginicus	Beach grass	0.12
Stenotaphrum secundatum	San Augustine	1.20

During one phase of a current study of tropical forage crops, the oxalate content of 24 frequently encountered forage and cut grasses was determined. All grasses were grown under similar field conditions in 10- by 25-foot plots and harvested at 6 to 10 weeks of age. In order to reduce the errors resulting from variation within each species, the sampling techniques developed and recommended by Vickery and Meiss (*6*) were used, with slight modifications. Each grass sample was a composite of 50 plants, including stems and leaves but no seeds. These samples were chopped, dried at 80°C in a forced-draft oven for 24 hours, and ground to pass an 80-mesh sieve. The oxalate concentrations were then determined by the permanganate method as modified and described in detail by Moir (*7*). The results of these analyses are shown in Table 1.

This brief chemical study brought out the fact that the most commonly used and highly recommended (*8*) grasses were those containing the highest concentrations of oxalates. Five widely used grasses contained more than 1.6 percent oxalic acid (Table 1). Extended and elaborate feeding trials by other investigators have shown that roughage containing 1.6 percent oxalic acid will lead to a negative calcium balance when it is fed *ad libitum* to nonlactating cows (*9*).

Whether or not the oxalate-rich grasses are a contributing cause to the low productivity of milk in the tropics is at present unknown. The percentage of the required digestive nutrients that can be obtained safely from oxalate-rich grasses has not been determined.

A detailed study of the chemical composition of one of the oxalate-rich grasses (Merker grass) is being made in conjunction with a feeding trial. This comprehensive knowledge of the chemical composition, together with the digestibility data, should indicate whether or not this oxalate-rich grass is defective in nutritive value or detrimental to the health of dairy cows (*10*).

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