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 electroosmosis can be eliminated by the use of suitable film thickeners (*2*). Further experiments are being conducted along these lines.

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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.

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.

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

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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Diffusion Constant and Diffusion Coefficient

J. Verduin has pointed out that the Krogh diffusion constant has been erroneously regarded as an index of diffusivity of gases in liquid media (*1*). The confusion has arisen from failure by some biologists (i) to examine the units used by Krogh (*2*) and (ii) to compare them with those of the true diffusion coefficient. For his concentration gradient, Krogh has used the difference in partial pressure of gas per unit length of liquid phase, thereby introducing solubility into his diffusion constant; this distinguishes it from the diffusion coefficient.

I join Verduin in criticizing those biologists who have made gross errors in their use of the Krogh diffusion constant. However, a number of points in Verduin's paper may be misleading and are therefore discussed here.

1) It is worth noting that the value of *D*, the diffusion coefficient, for O_2 in water at 16°C (1.607 cm^2/day) quoted by Spoehr (*3*) was not directly determined but was derived by Carlson (*4*) from his own experimental determination of 1.720 cm^2/day at 18.2°C. Carlson assumed a 3-percent change in *D* per degree change in temperature to derive a value for 16°C.

Verduin has misquoted the value calculated by Carlson and quoted by Spoehr. He quotes it as 1.607 cm^2/day at 20°C. As a consequence, Verduin's calculation of a Krogh diffusion constant at 20°C of 0.346 has no foundation.

2) The effect of temperature on the diffusivity of oxygen bears closer examination than that given by Verduin.

Table 1 summarizes, first, quoted values of *D* for O_2 in water (or dilute aqueous solutions) and second, values used for the increase in *D* with temperature for various substances in water.

Table 2 gives values for the increase in the diffusion coefficient of oxygen in water per degree rise in temperature. A linear increase per degree has been assumed and only the experimentally determined values of *D* from Table 1 have been used.

It appears from Table 2 that it is reasonable to suggest that the diffusion coefficient for oxygen in water increases linearly about 3 percent per degree rise in temperature within the range of 16° to 25°C.

The figure used by Verduin, 1.6 percent per degree Celsius (*15*), is a characteristic of the diffusion current measured in polarographic determinations and is dependent on a number of other variables in addition to the diffusion coefficient of the substance being examined.

3) Krogh asserts that his diffusion constant increased about 1 percent per degree rise in temperature. Verduin maintains that this statement is false and that there is no change in the Krogh constant within the temperature range of 20° to 30°C.

Table 1. Values of *D* and its change with temperature for O_2 in aqueous media

Temperature (°C)	Substance	Original measure of *D*	*D* (10^{-5} cm^2/sec)	Approx. linear increase in *D* (%/°C rise in *T*)	Authority
16	O_2	1.62 cm^2/day	1.875		Hüfner (*5*)
18	O_2		1.98	3*	Carlson (*6*)
18.2	O_2	1.720 cm^2/day	1.99	3*	Carlson (*4*)
22	O_2	2.22×10^{-5} cm^2/sec	2.22†	3*	Brdička and Wiesner (*7*)
25	O_2	2.38×10^{-5} cm^2/sec	2.38	‡	Kolthoff and Laitinen (*8*)
25	O_2	2.6×10^{-5} cm^2/sec	2.6		Kolthoff and Miller (*9*)
28 to 37	O_2			3*	Davies and Brink (*10*)
20	O_2			2.5*	Hill (*11*)
20 to 30	unspecified			2.5*	Höber (*12*)
20 to 25	unspecified			2.9*	Bull (*13*)
unspecified	unspecified			3*	Einstein diffusion equation (Höber, *12*)

* Values stated by authors but not experimentally determined. † Determined by dropping mercury electrode—mean value of six readings where drop time was 2 to 4 sec (see Kolthoff and Lingane, *14*). ‡ Diffusion current changed 4 percent per degree (*14*).

Table 2. Change in *D* with temperature calculated from values in Table 1

Temperature interval (°C)	Linear increase in *D* (%/°C rise in *T*)
16 to 18.2	2.8
16 to 22	3.1
16 to 25	3.0
16 to 25	4.3*
18.2 to 22	3.0
18.2 to 25	2.9
18.2 to 25	4.5*
22 to 25	2.4
22 to 25	5.7*

* The results based on Kolthoff and Miller's value $D = 2.6 \times 10^{-5}$ cm^2/sec at 25°C are of doubtful significance. Kolthoff and Lingane (*14*) in their latest book have used the earlier experimental value of 2.38×10^{-5} cm^2/sec at 25°C.

The decrease in the solubility of oxygen between 16° and 25°C is about 1.6 percent per degree. The relationship shown between the experimental determinations of four observers at four temperatures indicates an increase in *D* of about 3 percent per degree rise in temperature for oxygen within the same temperature range in water. Thus,

$$K_{T+1} = K_T \times \frac{103}{100} \times \frac{100}{101.6}.$$

That is,

$$K_{T+1} = K_T \times 1.014,$$

where K_T is the Krogh diffusion constant at temperature *T*.

Thus the increase in the Krogh diffusion constant is about 1 percent per degree rise in temperature within the range given.

4) Verduin has produced evidence of the misuse of the Krogh diffusion constant. Some physiologists have treated diffusion problems without confusion and it may be worth while recording some of them here for future reference. Hill (*11*) used both Krogh diffusion constants and true diffusivities, illustrating the interrelations of the two and their changes with temperature. Hill devoted some time to discussing the diffusivity of CO_2 in tissues (based on Krogh's value) which is still 1.2 times the diffusivity of O_2 when solubility differences are corrected. It is felt that the work of Hill has not received the attention it deserves; it has evidently escaped the notice of Prosser *et al* (*16*) and perhaps other physiologists. More recent biological studies of O_2 and CO_2 diffusion through tissues that clearly appreciate the nature of the diffusion coefficient include Briggs and Robertson (*17*), Roughton (*18*) and James (*19*).

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