phorylated, was not investigated in the course of this research.

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The Experimental Feeding of Parathion to Dairy Cows[1]

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The increasing use of parathion (O,O-diethyl, O,*p*-nitrophenyl thiophosphate) as an insecticide on forage crops has resulted in speculation as to its excretion in the milk of dairy cows fed residual amounts of the chemical. Consequently an experiment was designed to determine the presence or absence of parathion in the milk of dairy cows fed parathion in capsules.

Ten dairy cows in heavy lactation, and representing the Ayrshire, Jersey, Guernsey, and Holstein breeds, were divided into two groups and fed commercially available parathion in the form of a 25% wettable powder (analysis: 23.75% parathion) continuously for 81 days. Cows in one group were fed parathion in capsules at the level of 5 ppm of an estimated roughage dry matter intake of 2.25 lbs/100 lbs of body weight daily. Cows in the other group were fed parathion in capsules at a level of 1 ppm of the estimated roughage dry matter intake. The two feeding levels were equivalent to 0.11 mg of parathion per kg of body weight for the 5-ppm group and 0.02 mg of parathion per kg of body weight for the 1-ppm group. These feeding levels represent an intake of parathion greater than that which would be ingested as residues (less than 1 ppm) on forage crops treated with amounts of parathion necessary for good insect control (*3*, *5*). In order to study the effect of feeding parathion to cows in late lactation, an Ayrshire cow in late lactation was added to each of the above groups. These cows were fed parathion for only 2 weeks, at which time they were turned dry.

At the end of 81 days, all but 2 cows in the 5-ppm group were dropped from the experiment. At this time, the 2 remaining cows were administered parathion in amounts that were doubled each successive week until a parathion intake equivalent to 40 ppm of the roughage dry matter had been fed. At this final level they were receiving 0.88 mg of parathion per kg of body weight daily.

Samples of carefully mixed milk were taken on alternate days for 6 days prior to the beginning of the experiment and then on alternate days for 6 days subsequent to the beginning of parathion feeding, after which samples were taken at semiweekly intervals for 3 weeks. Thereafter, samples were taken once a week for the duration of the experiment.

Application of the sensitive colorimetric method of Averell and Norris (*1*) for the estimation of small amounts of parathion was tried on 100-g samples of milk, to which known amounts of parathion were added, and extraction attempts were made using the methods developed by Schechter *et al.* (*4*) and Carter (*2*). The presence of interfering substances and a very low recovery of the parathion added to the milk did not permit the use of either of the extraction methods. The difficulty seemed to lie with the development of a selective extraction procedure that could be used to separate the parathion from the milk. Upon the suggestion of the American Cyanamid Company, a procedure involving the use of a liquid-liquid extraction apparatus was tried; essentially this method involved a prolonged percolation of petroleum ether through a column of milk and ethyl alcohol. The mixture in the extraction chamber was stirred at ½-hr intervals with a wire stirrer inserted through the reflux condenser. Standard curves were prepared from data obtained from analyses of milk to which were added known amounts of parathion. Amounts ranging from 20 to 120 μg were added to 100-g samples of milk. The liquid-liquid extraction procedure was carried out for an optimum period of 6 hr, followed by the concentration of the petroleum ether and analysis by the Averell and Norris method. The percentage transmittance values were obtained using a wavelength setting of 555 mμ with a Coleman Model 14 spectrophotometer. It was found that milk blanks varied from 95% to above 100% transmittance when compared with the reagent blanks; therefore, in the actual analyses, no reading above 90% was considered significant. Technical petroleum ether (Skelly-solve "B") was used in all the analyses, since it was found that the use of purified petroleum ether did not improve the results. The use of 95% ethyl alcohol and distilled water to dissolve the residue remaining after the evaporation of the petroleum ether extract prevented the for-

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mation of a bothersome turbidity that developed when benzene was used. Any slight turbidity appearing upon the addition of water to the solution of the residue dissolved in ethyl alcohol either disappeared during the reduction process or was removed by filtration.

Approximately 250 separate analyses were made during the course of the experiment. At no time was any parathion found in the milk from any of the cows fed parathion. Biological assays of the milk from the parathion-fed cows, using adult houseflies (*Musca domestica* L.) were conducted, and the absence of mortality among the flies served to confirm the negative analytical findings. No objectionable flavor was noted in the milk of the cows fed parathion, and no harmful effects to the health of the cows have been observed.

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A Rational Method for Calculating Colloid Osmotic Pressure of Serum

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The usual equation for calculating the osmotic pressure of a solution is:

$$\pi = CRT \qquad (1)$$

in which π is the osmotic pressure, T is the absolute temperature, R is the ideal gas constant, and C is the concentration of the solution in moles of solute per liter.

Capillary endothelium is a dialyzing membrane permeable to ordinary ions but not to a colloid or its ion. Within the capillary is blood serum, and outside of it is interstitial fluid.

The symbols y and $y+z$ are respectively the sum of nonprotein anion and cation normalities in blood serum, and x the same for interstitial fluid. The normality of the serum protein is z, and n its valence. According to the Donnan equilibrium relation (*4*),

$$x^2 = y(y+z). \qquad (2)$$

Because of the complexity added by consideration of bivalent ions (*9*), their low concentration, and consequently, the small scale of their effect, we omit them from this discussion. The observed pressure of equilibrium is the difference between the total osmotic pressures of the two solutions (*10*), or

$$\pi = RT(z + z/n + 2y - 2x). \qquad (3)$$

[1] The suggestion of this problem by Joseph B. Kirsner is gratefully acknowledged. This paper was written while the author was with the Frank Billings Medical Clinic, Department of Medicine, University of Chicago.

The symbol π represents in equation (3) what is generally called the colloid osmotic pressure. Putting the expression for x obtained from equation (2) into (3), we have

$$\pi = RT[z + z/n + 2y - 2\sqrt{y(y+z)}]. \qquad (4)$$

Since albumin and globulin are the components of serum protein, let

$$z = z_A + z_G,$$

where z_A is the molarity of albumin and z_G the same for globulin. If the law of partial pressures applies,

$$\pi = RT[z_A/n + z_G/n + z_A + z_G + 2y - 2\sqrt{y(y + z_A + z_G)}]. \qquad (5)$$

In order to use equation (5), molecular weights for globulin and albumin as determined by ultracentrifugation (*2, 3, 5, 16*) may be utilized, choosing 70,000 for albumin and an average value of 165,000 for globulin. Because protein is a titratable anion, it has been possible to determine the amount of alkali necessary to neutralize a specimen of serum protein when the pH of the medium is known (*14*).

The above information is used to formulate the expression for Z below. Let

A = g of albumin per 100 ml of serum,
G = g of globulin per 100 ml of serum,
pH = serum pH,
$[HCO_3^-]$ = mEq./l of serum bicarbonate,
$[Cl^-]$ = mEq./l of serum chloride,
T = temperature in °C + 273,
R = 0.849 liter-mm H_2O/millimole-(°C + 273), and
organic acid (in serum, assuming its acidity due to univalent carboxyl groups) = 6 mEq./l (*7*).

Define

$$Y = 0.849([HCO_3^-] + [Cl^-] + 6) \qquad (6)$$

and

$$Z = 1.061A(\text{pH} - 5.16) + 0.654G(\text{pH} - 4.89). \qquad (7)$$

Then, if P is the colloid osmotic pressure of the serum, we have

$$P = T[0.1212A + 0.0514G + Z + 2Y - 2\sqrt{Y(Y+Z)}]. \qquad (8)$$

Equations (6), (7), and (8) stand in distinction to previously proposed empirical relations derived from curve fitting of a set of experimental data (*1, 6, 8, 12, 17, 18*). Scatchard (*15*) attempted a theoretical derivation, but made an erroneous substitution invalidating his result.

The impression exists that, in order to make a colloid osmotic pressure determination, a direct measurement with an appropriate osmometer should be carried out. To show that for clinical purposes this is not necessary, colloid osmotic pressures calculated with equations (6), (7), and (8) are compared with measured ones in Fig. 1.

If the effect of $[HCO_3^-]$ and $[Cl^-]$ on serum colloid osmotic pressure is known, the effect of serum sodium may readily be determined from the following equation:

$$1.178Z + [HCO_3^-] + [Cl^-] = [Na^+] + 4, \qquad (9)$$

remembering the normal concentrations of the more dilute ions, as well as the original omission of consideration of