Effect of Dietary Polysorbate 80 on the Serum Concentration of Calcium and Magnesium in the Rat

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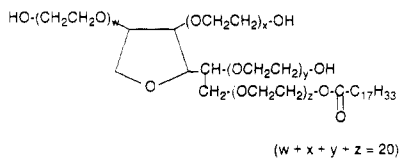
Test rats were fed a diet supplemented by 1% Polysorbate 80, and their serum was monitored for unbound and total calcium ion concentration, total magnesium ion concentration, and total unbound ion concentration. No significant difference compared to control animals was detected for the magnesium ion and total unbound ions concentrations, but significant drops in both the unbound and the total calcium ion concentrations were detected after 60 days. This effect was shown to be sex dependent. There was also no significant effect on weight gain during the experiment.

Polysorbate 80 (1), also known as Tween 80, is a common surfactant used extensively as a solubilizing agent in biochemical, medical, and pharmacological research. The



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assumption of many researchers has been that 1 is itself totally inert and does not interfere with living systems with which it comes in contact. It is also used in a varietty of foods, constituting up to 1.25% of many brands of ice cream; previous toxicological research (Gloxhuber, 1974) and experience have indicated that it is devoid of any gross deleterious effects when taken internally.

Previous research (Thoman, 1986) has shown that Polysorbate 80 is an effective ionophore, able to sequester and transport across a model membrane (CH_2Cl_2) a variety of ions, many of them biologically important (Ca^{2+}, Na^+, K^+). Such information raised the possibility that 1 might not be as inactive in living systems as sometimes supposed. The present research was initiated to determine whether dietary Polysorbate 80 has any effect on the concentrations of several important ions in the blood serum of rats.

EXPERIMENTAL PROCEDURES

Apparatus. Atomic absorption spectrographs were determined with a Perkin-Elmer Model 3030B.

Reagents. Mature Long Evans rats were purchased from the Pocono Rabbit Farm, Canadensis, PA. The solution of Cal-Red indicator (Patton and Reeder, 1956; Sadek and Reilly, 1959; Turekian and Bolter, 1966) was prepared by dissolving 0.1 g of the solid (from Rayflex Exploration Co.) in 100 mL of 95% ethanol; this was stored in a refrigerator in an aluminum foil covered flask. The EDTA solution contained 0.310 g of Na_2-EDTA in 500 mL of water. The standard calcium solution was made by dissolving 0.250 g of dry $CaCO_3$ in 6 mL of 1.0 N HCl and diluting it with water to 1.0 L. Amberlite 100 cation-exchange resin (hydrogen form) was obtained from Rohm and Haas and Polysorbate 80 from Fisher Scientific; both were used without further purification.

(1) *Determination of Unbound Ions.* Six Long Evans rats (three male, three female) were fed a normal diet, while six others (three male, three female) were fed the same diet plus approximately 1% by weight of Polysorbate 80 (equivalent to about 560 mg/kg body weight) in 0.5 mL of honey, given daily by gavage. After 60 days, blood was drawn from each rat by penetration of the orbital sinus. Serum was obtained by allowing blood to clot and then centrifuging it for 20 min at 21 000 rpm in a refrigerated centrifuge.

(A) *Unbound Calcium Ion Determination.* The following procedure is first done with the standard calcium solution. A micropipet is used to transfer 0.1 mL of standard (5 mequiv/L) calcium solution to a position on a spot plate. After addition of two drops of 2.0 N KOH and two drops of Cal-Red indicator solution, EDTA solution is added with a micropipet to an end point (change from red-purple to light blue). The procedure is repeated using 0.1 mL of serum to a green rather than a light blue end point. Each sample is analyzed in triplicate to establish precision.

(B) *Total Unbound Cation Determination.* Amberlite 100 cation-exchange resin (2.5 g) is placed in a 25-mL buret and 0.2 mL of a 0.15 M aqueous solution of NaCl is added, followed by two 5-mL washings with water. After draining is complete, two drops of Phenol Red indicator solution is added to the eluant, which is then titrated using a micropipet to an end point (orange to bright pink) with 0.2 M NaOH. Serum samples (0.2 mL) are analyzed in the same way. The columns may be regenerated between runs by washing with about 10 mL of a 4% HCl solution, followed by water until the eluant is neutral. A fresh column is made after six analyses. Determinations are again made in triplicate.

(2) *Determination of Total (Bound and Unbound) Ions.* Twelve Long Evans rats (6 male, 6 female) were fed a normal diet, and 12 others (6 male, 6 female) were fed the same diet plus approximately 1% by weight of Polysorbate 80 (equivalent to about 560 mg/kg of body weight) dissolved in 0.5 mL of honey, given daily by gavage. After 60 days, serum was obtained as described in the previous experiment and analyzed, using specific ion electrodes, by atomic absorption spectroscopy (Willis, 1960a-c; Gimblet et al., 1967). Calcium was determined at a wavelength of 422.7 nm and magnesium at 285.2 nm, both in the presence of 0.1% w/v of lanthanum chloride, using a mixture of air and acetylene.

RESULTS AND DISCUSSION

The results of the first experiment, which determined the serum concentration of unbound calcium ions and total unbound ions, are summarized in Table I. The probability

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Table I. Effect of Dietary Polysorbate 80 on Unbound Calcium Ion and Unbound Total Cation Concentrations in the Serum of Rats[a]

	unbound $[Ca^{2+}]$, mequiv/L	unbound [total cations], mequiv/L
male		
control	4.58 ± 0.03	153 ± 9
test	4.31 ± 0.04	151 ● 5
% change	−5.90	−1.3
P	<0.001	NS[b]
female		
control	4.67 ± 0.08	134 ± 15
test	4.27 ± 0.07	127 ± 3
% change	−8.57	−5.2
P	<0.001	NS

[a] Twelve Long Evans rats, control (three male, three female) and test (three male, three female). [b] NS, not significant.

Table II. Effect of Dietary Polysorbate 80 on Total Calcium and Magnesium Ion Concentrations in the Serum of Rats[a]

	total $[Ca^{2+}]$, mequiv/L	total $[Mg^{2+}]$, mequiv/L
male		
control	5.98 ± 0.26	0.65 ± 0.09
test	5.46 ± 0.35	0.61 ± 0.12
% change	−8.70	−6.15
P	<0.001	NS[b]
female		
control	6.69 ± 0.26	0.71 ± 0.14
test	5.99 ± 0.12	0.67 ± 0.12
% change	−10.46	−5.63
P	<0.001	NS

[a] Twenty-four Long Evans rats, control (six male, six female) and test (six male, six female). Monitored by AA spectroscopy. [b] NS, not significant.

of there not being a significant difference between the calcium ion concentrations of test animals compared to controls was less than 1 in 1000 for both males and females, with an average drop of 7.2%. The effect, however, seems to be sex related, being significantly stronger in the females. In contrast, no significant effect was indicated in total unbound cations; the slight drop of concentration in the test animals might be attributed to the loss of unbound calcium ions.

Table II contains the results of the second experiment. In this case, total calcium and magnesium ion concentrations were monitored by atomic absorption spectroscopy. The test animals suffered a significant drop in the serum calcium ion concentration but not in their serum magnesium ion concentration, and again the effect of the calcium ion loss was more pronounced in the females than in the males. The average loss was up to 9.6%, with an equally impressive level of significance.

Considering the importance of calcium ions in a variety of biochemical processes, the significance of these findings regarding calcium ion depletion is evident. Further and more extensive experimentation with calcium ions, as well as with the other important biochemically active ions (notably sodium and potassium ions) is certainly indicated. If these results are confirmed, certain persons (e.g., those at risk of osteoporosis) might have to be advised to avoid foods (e.g., ice cream, salad dressings, cheese spreads) that often contain Polysorbate 80.

There are two collateral findings connected with these studies. During the course of the second experiment, the rate of weight gain of the animals was monitored, but no significant difference was detected between test and control animals or between males and females.

At the end of the first experiment, an experienced technician drawing the blood from the optical sinuses of the animals remarked early on that some of the animals had noticably lower blood pressure than the others. The technician did not know which animals were test or control, but those animals subsequently cited as having lower blood pressure were invariably test animals. We hope to determine any connection that may exist between dietary Polysorbate 80 and the blood pressure of rats in the near future.

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