The rise in blood cholesterol with cholesterol feeding showed the usual individual variation, but was roughly comparable in the 3 groups fed. During the third month when cholesterol had been stopped, the blood cholesterol began to fall promptly in the rabbits that did not receive KI, while, in contrast, there was an actual rise in the average blood cholesterol for the group given KI.

It is a common experience to find that certain rabbits in a group fed cholesterol fail to develop a hypercholesterolemia. There has been no explanation for this resistance. The high incidence of this in the present series was unusual. Thus, of 62 rabbits fed cholesterol, 17 failed to develop a hypercholesterolemia. A point of possible significance was that 13 of the 17 animals were males, a ratio of 3:1, whereas in the total group of 62 rabbits only 30, or approximately one-half, were males.

Atheromata. Gross aortic lesions were present in 8 of the 15 animals killed after 2 months of cholesterol feeding, in 6 of the 14 that received KI during the third month, and in 11 of the 16 that received no KI. The period of feeding by this method was too short to attach much significance to these results.

Conclusions. (1) The cholesterol content of rabbit liver, increased by cholesterol feeding, tends to return spontaneously to a normal value when the feeding is stopped. KI apparently does not accelerate this fall. (2) The normal decrease in adrenal weights and the prompt fall in the blood cholesterol occurring when cholesterol feeding is stopped are both inhibited by the administration of KI at the conclusion of the feeding period. (3) The high incidence of males among rabbits failing to develop a hypercholesterolemia with cholesterol feeding warrants further study.

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The Deterioration of Vitamin D in Aqueous Solutions.

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The vitamins may be generally grouped into two classes, namely: (1) those soluble in fats and (2) those soluble in aqueous solutions. Vitamins A, D, and E are found nearly exclusively in association with fats, and vitamins B and C with substances soluble in water.

The reason for the distribution of vitamins in certain vehicles has not been definitely explained, although it may be readily surmised that specific vitamins probably deteriorate in solutions other than those in which they are found in nature. We have encountered such deterioration of vitamin D when emulsified in water.

Shelling and Tidwell[1] prepared oil-in-water emulsions of viosterol, which were miscible in water and in milk. They suggested that such emulsions offered a means by which larger amounts of vitamin D might be added to milk than was possible to impart by direct irradiation of the milk or by feeding vitamin D preparations to the cow. Subsequent clinical experiments[2] revealed that the *emulsified* oily solutions of vitamin D, when added to milk, were more than 10 times as potent as viosterol in oil, not so treated. Thus, in a study of *134* cases of severe rickets studied by Shelling and Hopper,[3] many of which were partially or entirely refractory to ordinary doses of cod liver oil, at least 20 to 30 drops of viosterol were required to heal the rickets completely in an average time of 3.7 months. On the other hand, with emulsions of viosterol, severe forms of rickets may be healed with as little as 1.5 drops of viosterol (125 Steenbock or 350 International units) in an average time of about 3 months.

In the first few months of the experiment, several rachitic children were given the emulsion and healing took place as expected, but after that time healing occurred very slowly and, in some instances, not at all. When a new emulsion was fed to the latter group, healing took place promptly, as in the first group. Bioassays of the old emulsion, made in November, 1933, revealed the fact that it had lost nearly all its potency on standing in the icebox in brown, tightly stoppered bottles for a period of 5 months. The experiment was repeated with the new emulsions and these too showed progressive deterioration. The emulsion, which originally contained 5% of viosterol 250-D, or 500 International units per gram, assayed only 280 units after 3 weeks, 125 units after 6 weeks, and less than 40 units per gram, after 6 months. Some samples showed no potency at all at the end of this period. The containers were opened from time to time and no effort was made to exclude air. The loss of potency was only slight when the emulsions were kept in sealed glass vials, in which the oxygen was replaced by nitrogen. Bioassay of these emulsions after 4 months' standing showed an average

[1] Shelling, D. H., and Tidwell, H. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 605.

[2] Shelling, D. H., and Hopper, K. B. (To be published).

[3] Shelling, D. H., and Hopper, K. B., *Bull. Johns Hopkins Hosp.*, 1936, **58**, 137.

of 400 as contrasted with 500 International units per gram initially present. The slight deterioration in this case may have been due to the exposure of the emulsion to the atmosphere, while it was being fed to the rats, or to some other unknown factor.

Some of the emulsions were sent to Dr. Charles E. Bills, of the Research Laboratories, Mead Johnson and Company, Evansville, Indiana, who corroborated these findings.

Similar loss of potency of pure vitamin D in crystalline form was noted by Fuchs and Van Niekerk;[4] and a quantitative difference in the potency between freshly prepared water and milk solutions of Drisdol (vitamin D in propylene glycol), stored for months at a low temperature, was observed by Supplee and his associates.[5]

The exact mechanism of the deterioration of vitamin D in states other than oil is not definitely known. It is highly probable that it is due to the relatively high solubility of oxygen in water, in which case the compound may be oxidized more rapidly than in oil solutions. In this respect the stability of vitamin D differs from that of vitamin A, since Hopkins,[6] Mellanby[7] and McCollum[8] have found the latter to be destroyed by bubbling oxygen through heated butter fat or cod liver oil. In Mellanby's and McCollum's experiments the potency of vitamin D in the cod liver oil was apparently not impaired by this procedure.

The gradual loss of potency of vitamin D in aqueous solutions raised the question as to its stability in canned evaporated milk. Accordingly, we have prepared, with the aid of the Research Staff of a dairy producing canned milk, a vitamin D milk in cans, by emulsifying a known quantity of viosterol with a measured amount of milk. The milk fat from sample cans was assayed from time to time and was found to contain the calculated amount of vitamin D, within the errors of bioassay, and when fed to 6 children with very severe rickets, in amounts ranging from 240 to 350 International units (1 to 1.5 drops of viosterol in oil), the rickets healed promptly and completely within a period of from 3 to 4 months.

Inquiry from the Wisconsin Alumni Research Foundation and from several manufacturers of canned, irradiated milk brought the reply that they found such milk stable, with respect to vitamin D

[4] Fuchs, L., and van Niekerk, J., *Biochem. Z.*, 1935, **272**, 32.

[5] Supplee, G. C., Ansbacher, S., Bender, R. C., and Flanigan, G. E., *J. Biol. Chem.*, 1936, **114**, 95.

[6] Hopkins, G., *Biochem. J.*, 1920, **14**, 725.

[7] Mellanby, E., Spec. Rep. No. 61, Med. Res. Council, London, 1921.

[8] McCollum, E. B., Simmonds, N., Becker, E. J., and Shipley, P. G., *J. Biol. Chem.*, 1922 **52**, 293.

potency, even after standing for more than a year. It is interesting that, although most canned milk is not "vacuum packed", most of the air is removed from it before sealing by the process of warming and condensing, and the removal of most of the oxygen by this means may be responsible for the stability of vitamin D in canned milk.

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Toxicity of Chlorazol-fast-Pink in Dog Fish.

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Chlorazol-fast-pink, one of the azodyes,[1, 2, 3] has been found an effective anticoagulant *in vivo*. In the effort to avoid clotting in arterial cannulae in some pharmacological studies of the circulation of the dog fish, the author has attempted to use this substance. Huggett and Rowe give its M.L.D. for rabbits as 300 mg. per kilo and Feldberg has found that in dogs 80-100 mg. per kilo in 8% solution given intravenously did not exert any deleterious action on heart or vessels. However, the author in some 20 experiments found that the intravenous injection of as little as 20-30 mg. per kilo of this dye in 3% solution regularly depressed the heart and the blood pressure markedly and that about 100 mg. per kilo was a lethal dose.*

This report is made in the belief that it may be of value to any one contemplating the use of this dye in fishes.

1 Parkes and Brambell, *J. Physiol.*, 1932, **74**, 65.

2 Huggett and Rowe, *ibid.*, 1933, **80**, 95.

3 Feldberg and Guimaris, *ibid.*, 1936, **86**, 306.

* The preparation used was the same (out of the same vial) which in the Department of Pharmacology at Tulane had been entirely satisfactory when given intravenously to cats and dogs in dosage of from 80 to 100 mg. per kilo.