

In striking contrast to the emulsion-fed animals were those receiving the same amount of cod liver oil supplied as plain oil alone or as plain oil + the concentrated malt extract fed separately. Several of the animals in each of these groups died before the end of the 35-day test period, while those that did live made poor growth or lost weight and were in poor physical condition, with more or less severe xerophthalmia at the end of the period.

From the results there can be little doubt but that the vitamin A present in the oil-malt extract emulsion is much better utilized than that of the plain cod liver oil.

Although the plain cod liver oil + the malt extract fed separately showed no better results than the plain oil alone, thus tending to discount the idea of the synergistic action postulated above, there is still the possibility that such an action may function only when the extract and oil are intimately mixed, *i. e.*, in the emulsified form. Unless it can be shown, however, that the malt extract *per se* plays some part in increasing the value of the emulsified oil, it would appear that the logical explanation for the better results with the emulsified product is that the very fine subdivision of the oil favors a more complete absorption and utilization within the animal body.

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Blood Cholesterol in Dogs on an A Deficient Diet.

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Drummond¹ showed that vitamin A was linked to cholesterol and could be separated from it without losing its vitaminic activity. No mention was made that vitamin A might affect the metabolism of cholesterol. Liang and Wacker² found that the total cholesterol in rats fed a diet low in vitamin A, fat and cholesterol was greater than in a group of animals fed a diet containing butter and fat. They felt that these results showed that the metabolism of cholesterol was

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¹ Drummond, J. C., *J. Soc. Chem. Ind.*, 1924, **43**, 928. Drummond, J. C., and Coward, K. H., *J. Soc. Chem. Ind.*, 1922, **41**, 561.

² Liang, B., and Wacker, L., *Biochem. Z.*, 1925, **164**, 371.

affected by vitamin A. No blood cholesterols are reported. Wieland and his associates³ suggested that in the normal animal cholesterol is broken up by vitamin A to bile acids and secreted in the bile.

While studying the effect of A deficiency in young and adult dogs we noted a rise in the blood cholesterol during the period when the symptoms of A deficiency were present. The A deficient diet consisted of cracker meal 100 to 150 gm., lard 20 to 30 gm., vitamins B, G, and D in the form of irradiated yeast and 3 gm. of a salt mixture.⁴ In addition the 2 young dogs, 68 and 69, received 100 cc. of boiled milk. When the symptoms of the deficiency were well advanced dog 12 was given carotene and dog 72 was given cod liver oil in the form of the concentrate. The carotene, obtained from the Mead Johnson Laboratories, was put up in maize oil and had an antixerophthalmic potency 10 times that of cod liver oil. The cod liver oil tablets, supplied by the White Laboratories, contained 1000 units of vitamin A. Of the 4 dogs observed, one, 68, died on the 21st day of the diet. Dog 69 was killed on the 41st day of the diet, at which time she showed loss of appetite, listlessness and loss of weight. Dog 12 was killed after having received carotene for 66 days. The livers of dogs 12, 68, and 69 were analyzed for their vitamin A and carotene content. Extraction of the liver oil was done by the method reported by Moore.⁵ Determination of the vitamin A content was done by the Carr and Price⁶ modification of the Rosenheim and Drummond antimony trichloride test. We have reported the vitamin A and carotene content of the livers of well-fed laboratory dogs.⁷ Compared to such animals the vitamin A content of dog 12, fed carotene, is about 1/6 that of the normal animals. The vitamin content of the livers of dogs 68 and 69 was very low. The blood cholesterols were done by the Bloor method.⁸

Table I shows the blood cholesterol findings on 2 young dogs, 68 and 69. In both animals there is a sharp rise which is maintained during the entire A deficient period. In dogs 12 and 72 there is also a sharp rise in the blood cholesterol (Tables II and III) but

³ Wieland, H.; Wieland, H., and Mothes, W.; Wieland, H., and Schlichting, O., *Z. f. physiol. Chem. Bd.*, 1924, **134**, 140, 149, 276.

⁴ Cowgill, G. R., *Am. J. Phys.*, 1921, **56**, 420.

⁵ Moore, T., *Biochem. J.*, 1930, **24**, 692.

⁶ Carr, F. H., and Price, E. A., *Biochem. J.*, 1926, **20**, 497.

⁷ Ralli, E. P., Pariente, A., Flaum, G., and Waterhouse, A., *Am. J. Phys.*, in press.

⁸ Bloor, W. R., *J. Biol. Chem.*, 1916, **24**, 227.

TABLE I.

| No. days on A defi- cient diet | Blood Chol. mg. | Symptoms of A deficiency |
|--------------------------------------|--------------------|---|
| Dog No. 68. Young Dog. | | |
| Aver. Bld. Chol. prior to diet | 185 | None |
| 13 | 303 | Loss of appetite |
| 14 | | Dog began coughing |
| 18 | | Discharge from eyes |
| 19 | 300 | Cough pronounced, listless, discharge from eyes profuse |
| Dog No. 69. Young Dog. | | |
| Aver. Bld. Chol. prior to diet | 223 | None |
| 3 | 262 | " |
| 19 | 303 | " |
| 26 | 317 | Loss of appetite for past 4 days |
| 27 | 370 | Dog less lively |
| 29 | 370 | Appetite slightly better |
| 33 | 370 | Loss of appetite more marked |
| 36 | 317 | Refused food. Fed by force |
| 40 | 317 | Quiet. Listless. |

TABLE II.

Dog No. 12. Full grown female. Average blood cholesterol prior to diet, 205 mg.

| Day of A def. diet | Blood Chol. mg. | Symptoms of A deficiency present |
|--------------------|-----------------------|--|
| 106th | 303 | Listlessness, loss of appetite, loss of hair, discharging sores over body. Loss of weight. |
| 108th | 247 | Symptoms same. Refused food for 2 days. |
| Carotene Period | | |
| Carotene cc. | No. days administered | Blood Chol. mg. |
| | | Effect on Symptoms of Deficiency |
| 1.25 | 1 | 254 |
| | 12 | 153 |
| | | No change |
| | | Greatly improved for 6 days |
| 1. | 4 | 175 |
| | 8 | 185 |
| | 12 | 174 |
| | 15 | 194 |
| | | Gained 1¼ kg. Coat improved |
| | | Improvement continued throughout this period. Appetite excellent. |
| .5 | 3 | 289 |
| | 6 | 260 |
| | 13 | 256 |
| | 16 | 310 |
| | | Sores reappeared under rear paws. Dog otherwise well |
| | | Sores gradually improved |
| | | Dog continued well and lively Gained ¾ kg. |
| .25 | 7 | 285 |
| | 13 | 271 |
| | | Dog apparently well. Coat still slightly shaggy |

following the refusal of food there was in each dog a moderate fall. In both these animals when carotene and cod liver oil were given there was definite improvement in the symptoms of A deficiency and a marked drop in the blood cholesterol. In dog 12 when

the amount of carotene was reduced the blood cholesterol rose again. Apparently, although the dog had enough of the vitamin to overcome the more marked symptoms of the deficiency she did not have enough to keep the blood cholesterol within normal limits. This is possibly another example of what Sherman⁹ refers to when he points out that vitamin deficiencies exist in various degrees. Between an abundance of any of the vitamins in the body and enough to keep the symptoms from being obvious there is plenty of opportunity for the less evident effect of avitaminosis.

TABLE III.

| Dog No. 72. Adult female. Average blood cholesterol prior to diet, 198 mg. | | |
|--|-------------------|--|
| No. days on A def. diet | Bld. Chol. mg. | Symptoms of A deficiency |
| 2 | 196 | None |
| 5 | 202 | |
| 7 | 224 | |
| 9 | 226 | |
| 11 | 215 | |
| 13 | 247 | Appetite poor. Loss of weight. From this time on the appetite was capricious, the dog eating only part of each day's meal. The animal was still lively |
| 15 | 228 | |
| 18 | 212 | |
| 20 | 226 | |
| 22 | 206 | |
| 25 | 222 | Fed forcibly |
| 27 | 208 | |
| 29 | 204 | |
| 31 | 215 | |
| 36 | 206 | |
| 38 | 221 | Appetite slightly better Hair definitely falling from extremities |
| 41 | 226 | |
| 44 | 226 | |
| 48 | 333 | Symptoms pronounced. Sores discharging. Skin scaly Pronounced loss of hair. Animal listless Sores much worse, breaking down. Eyes discharging |
| 52 | 303 | |
| 58 | 278 | |
| No. days on C. L. O. | | Cod Liver Oil Concentrate Started. 10 tablets daily |
| 5 | 219 | |
| 8 | 185 | |
| 13 | 188 | |
| 15 | 190 | |

The question, of course, arises as to whether the blood cholesterol might not fall, regardless of the administration of vitamin A. In No. 72 there was a drop of 55 mg. during the 10 day period when the symptoms were becoming worse. Cod liver oil caused a further drop of 93 mg. in 8 days during which time the dog's condition improved. In dog 12, during the time when she refused food, the

⁹ Sherman, H. C., *J. Am. Med. Assn.*, 1931, **97**, 1425.

cholesterol fell 56 mg. After 12 days on carotene, during which time the animals ate well and the symptoms improved markedly, the cholesterol fell 101 mg. In this animal reduction of the amount of carotene was accompanied by a rise in blood cholesterol. The hypercholesteremia apparently occurs while the animal is being depleted of its vitamin stores. When this depletion is advanced the blood cholesterol falls. It may well be that when the animal reaches a state of nutritive failure due to the deficiency and enhanced by the refusal to eat, the blood cholesterol falls because the animal is utilizing its body stores as a source of food. In young animals, dying of an intercurrent infection before profound nutritive failure can occur, the fall in blood cholesterol may not manifest itself. It seemed significant to us that the blood cholesterol was reduced rapidly to normal limits by the administration of the vitamin and the general condition of the animal improved at the same time.

Summary. Observations are reported on the level of the blood cholesterol in 4 dogs while on an A deficient diet. There is a rise in the blood cholesterol at the time when the animals developed the symptoms of A deficiency. In the young dogs this was maintained at a higher level than in the 2 older dogs. The feeding of vitamin A or its precursor caused a drop in the cholesterol. When the source of the vitamin was diminished the blood cholesterol rose. While the amount of the vitamin was adequate the blood cholesterol remained at the normal level.

Conclusion. These observations tend to substantiate the suggestion of other^{2, 3} investigators that the metabolism of cholesterol is affected by the amount of vitamin A in the body.

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Growth-Promoting Rachitogenic Diets for Rats.

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In the Medical Research Council's 1924 "Report on the Present State of Knowledge of Food Accessory Factors" emphasis is laid on the fact that rickets develops in well nourished children and that rachitic dogs also grow at a normal rate while the disease develops.