the warmer season of the year the time required for development is shorter than during the colder season. With decreasing temperature the time required for development increases and *vice versa.*

In summary, the fleas, *Ctenocephalides canis, C. felis* and *Pulex irritans,* collected from dogs harboring *Dirofilaria immitis,* have been found naturally infected with the microfilarial and larval stages of this parasite. The female fleas seemed much more susceptible to the infection than the males. All 3 species of fleas have been experimentally infected and in all 3 the extra-mammalian phase of the life cycle has been completed. The time required for this development in fleas was 120 hours in warm weather and 216 hours in cold weather. This phase of the life cycle was more rapidly achieved in fleas than in mosquitoes. It appears that both biologically and epidemiologically fleas are more suitable intermediate hosts of *Dirofilaria immitis* than had been previously supposed.

11228 P

Hyperthyroidism and Liver Function in Relation to B Vitamins.

VICTOR A. DRILL AND HARRY W. HAYS. (Introduced by W. W. Swingle.)

From the Biological Laboratory, Princeton University, Princeton, N. J.

Many authors have reported that the liver becomes altered both structurally[1] and functionally[2] during hyperthyroidism. One of the most marked changes in the liver during the experimental and clinical hyperthyroid states is a depletion of the liver glycogen.[1, 3] A high carbohydrate diet is usually given to hyperthyroid patients in an effort to promote glycogen storage in the liver.

Abelin[4] and Abelin, *et al.*,[5] showed that large amounts of casein, egg yolk, or yeast would lessen the liver damage of the thyroid-fed animals, and Drill[6] was able to prevent a fall in the liver glycogen of

1 *cf.* Frazier, C. H., and Brown, R. B., *Trans. Am. Assn. for Study of Goiter,* 1935.

2 Maddock, W. G., Pedersen, S., and Coller, F. A., *J. Am. Med. Assn.,* 1937, **109**, 2130. *cf.* reference1.

3 John, H. J., *J. Am. Med. Assn.,* 1932, **99**, 620.

4 Abelin, I., *Biochem. Z.,* 1930, **228**, 165.

5 Abelin, I., Knochel, M., and Spiehtin, W., *Biochem. Z.,* 1930, **228**, 189.

6 Drill, V. A., *J. Nutrition,* 1937, **14**, 355.

thyroid-fed rats by feeding large amounts of yeast. It is also known that the dietary requirements for vitamin B_1 and the B_2 complex are above normal during experimental hyperthyroidism in rats.[7] Since yeast can maintain the liver glycogen of thyroid-fed rats we decided to investigate any possible relationships between the liver function of hyperthyroid dogs and the yeast content of the diet. No studies of the liver function of hyperthyroid animals are reported in the literature.

Methods. Throughout the experiment the dogs received a modified form of Cowgill's casein diet No. III,[8] in which 21% lard was used, and the butter was replaced by 4% of cod liver oil, so that a known amount of vitamins A and D were added to the diet. The dogs used were all full-grown males weighing between 9 and 17 kg. The dogs were fed a stock diet of Purina checkers *ad libitum*. Two weeks before the thyroid feeding was started the dogs were placed on the modified Cowgill diet. The dogs were allowed to eat as much as they wanted for a 3-hour period each day. Each dog received a daily supplement of yeast No. 17800 in the proportion of 2 International units of vitamin B_1 per pound of body weight. The yeast contained 23 I.U. of vitamin B_1 per gram and 20 Sherman-Borquin units of vitamin G (flavin) per gram.* This makes the diet normal in all respects. The thyroid used was Lilly's desiccated thyroid gland, U.S.P.

The bromsulphalein method[9] was used to determine the liver function of the dogs. Five mg of dye per kg of body weight was injected intravenously into the jugular vein of unanesthetized trained dogs, and 30 minutes later 10 cc of blood was withdrawn under oil from the opposite jugular and the concentration of dye remaining in the serum determined. In the standards 4 mg of bromsulphalein per 100 cc of dilute NaOH was used as 100%.

Results. The 2 control dogs, receiving only Cowgill's diet and the daily yeast supplement, showed normal liver functions throughout the experiment. The dye retention in the serum of the controls at the end of 30 minutes was always less than 10%, generally being between 2 and 8%. Any retention of dye above 15% in the thyroid-fed animals was therefore considered abnormal and definite evidence of impaired liver function.

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7 Drill, V. A., and Sherwood, C. R., *Am. J. Physiol.*, 1938, **124**, 683.

8 Cowgill, G. R., *Am. J. Physiol.*, 1928, **85**, 45.

* The authors wish to thank Dr. H. W. Rhodehamel of Eli Lilly and Co. for supplying the large amount of desiccated thyroid gland that was required, and also Dr. C. N. Frey of the Fleischmann Laboratories for supplying the analysed yeast.

9 Rosenthal, S. M., and White, E. C., *J. Am. Med. Assn.*, 1925, **84**, 1112.

Dogs fed 0.6 gm thyroid gland/kg of body weight. Two dogs (Nos. 3 and 11) were fed this dosage. Dog No. 3 showed a normal liver function up to the 42nd day of thyroid feeding. On the 42nd day the yeast was reduced to one-half of its amount in the diet and 24 hours later the liver function showed 25% of dye retention. On the 44th day the yeast was removed from the diet and 24 hours later the test showed 150% retention of dye. More than 100% retention of dye is due to the fact that the colorimeter standards developed originally for the injection of 2 mg of dye per kg of body weight, have been retained even though a 5 mg dose is used. It is difficult to differentiate the color with higher standards.[2] The dye retention then dropped and remained at an average of 50% during the next 10 days. Dog No. 11 showed a marked drop in appetite after the 37th day of thyroid feeding. This indicated that the vitamin stores, in particular vitamin B_1, of the body were lowered and that now more vitamins were being metabolized than were being supplied in the normal diet. A liver function on the 41st day gave 45% retention.

Dogs fed 0.4 g of thyroid gland/kg of body weight. Dogs Nos. 2, 9, and 10 received this dosage. Dog No. 2 gave normal liver functions up to the 49th day of thyroid feeding. A test on the 59th day showed 25% retention. The yeast was then removed from the diet and 24 hours later 125% retention of dye was obtained. This dropped and remained between 25-40% during the next 10 days. Dog No. 10 showed similar results. Dog No. 9 gained a considerable amount of weight after the experiment had started, so that on his new weight basis he was receiving less than 0.4 g of thyroid/kg of body weight. His food intake was still above normal, and was not dropping, indicating that no depletion of the body stores of vitamins had occurred. On the 57th day his liver function was still normal, and no fall in appetite was observed. The yeast was removed from the diet on the 57th day and 24 hours later only 20% of dye retention was observed, which remained at this low level during the next 5 days. This shows that without vitamin depletion, as shown by the high food intake, that only a slightly abnormal liver function was obtained when the yeast was removed.

Discussion. The results indicate a definite relationship between the yeast content of the diet and the liver function of thyroid-fed animals.

Some of the dogs maintained normal liver functions for 40-50 days on the above diet while receiving thyroid gland, and then showed a marked abnormal liver function as soon as the yeast was removed. In the others the liver function became abnormal, without removing the yeast from the diet, in about 40 to 50 days. These

results are best obtained with a dosage of 0.6 g of thyroid gland per kg of body weight. It is known that the amount of vitamin B_1 in rat tissues is reduced by thyroid feeding,[10, 11] and that the thyroid-fed rat also requires more of the vitamin B_2 complex than is supplied in a normal diet.[7] We have found this same dietary relationship to be present in the dog.[12] This indicates that the abnormal liver functions in the thyroid-fed dogs are related to an increased requirement for some of the B vitamins, with a probable loss in body stores, as judged by the loss of appetite. This will be reported later in greater detail. A subnormal amount of the B vitamins in the diet may be at least partially responsible for the abnormal liver function that is observed in human hyperthyroidism.

The results of these experiments do not mean that the B vitamins are the only factors related to the production of abnormal liver function in hyperthyroid animals. However, this is the first causal relationship to be established for the production of abnormal liver function in hyperthyroidism. It is not yet known if a large amount of yeast in the diet can maintain a normal liver function in thyroid-fed dogs over a long period of time. Further experiments are in progress.

Conclusions. 1. Using a standard diet, with yeast of a known vitamin content, the liver functions of dogs was studied at two levels of thyroid feeding. 2. The production of the abnormal liver function in hyperthyroid dogs bears a causal relationship to the yeast in the diet.

11229

X-radiation and Growth Substances as Affecting Plant Primordial Tissues.

VIRGINIA C. IRVINE.* (Introduced by Francis Ramaley.)

From the Department of Biology, University of Colorado, Boulder, Colo.

Since little attention has been given to the primordial regions of plants as influenced by X-radiation and chemical growth-promoting substances the author has carried out experiments to determine effects of these agencies on growing points. Snow[1] observed that hetero-

10 Drill, V. A., *Am. J. Physiol.*, 1938, **122**, 486.

11 Peters, R. A., and Rossiter, R. J., *Biochem. J.*, 1939, **33**, 1140.

12 Drill, V. A., unpublished work.

* University Scholar in Biology.

1 Snow, M. and R., *New Phytologist*, 1937, **36**, 1.