

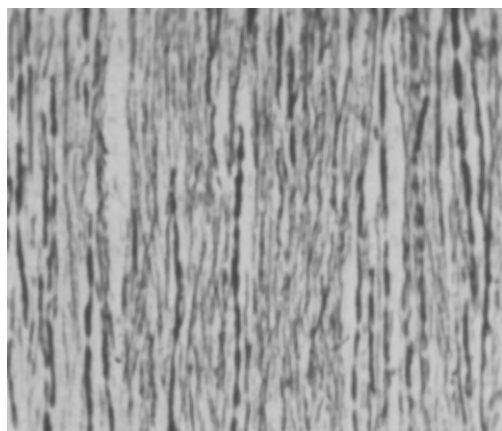


FIG. 2.

Detail from Fig. 1, showing the regenerated nerve in the zone of the graft. $\times 230$.

before treatment do not seem superior to undegenerated ones. Details will be reported later. Sclerotic islands present in a few grafts may tentatively be ascribed to imperfections in the freezing or rehydration process.

Most devitalized heteroplastic grafts behave like foreign bodies. However, masses of nerve fibers use their oriented surface as pathway.

The results indicate that the biophysical and biochemical benefits to regenerative nerve growth inherent in peripheral nerve are essentially preserved in the freezing-drying process. The fitness of devitalized nerve as graft brings the supply problem in nerve grafting nearer its solution. Banks of assorted nerve sizes stored in the dry condition could readily fulfill a steady demand.

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Hepatic Injury in Dogs Resulting from a Vitamin B Complex-Free Diet.

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Various authors have studied the effect of administration of the B vitamins in cases of hepatic cirrhosis,^{1,2,3} and experimental studies on the relation of the B vitamins, choline, methionine and cystine to hepatic damage have been reported.⁴⁻⁹ In this paper prelim-

inary studies were made on the effect of a vitamin B complex-free diet on the hepatic function of dogs.

Methods. Seven adult dogs, weighing between 7.9 and 14.3 kg, were used as experimental animals. When first obtained they were placed on a diet of Purina chow, and 2 weeks before the experiment was started they were fed on a modified form¹⁰ of Cowgill's casein diet No. 3,¹¹ plus a daily yeast supplement. The diet used was free of the B vitamins, the only source of these vitamins in the diet being supplied by the daily yeast supplement. The dogs had access to their food for a 3-hour period each day. The amount of food eaten was measured. Following control measurements of liver function, the yeast was removed from the diet of 5 of the dogs and the liver function, food intake and weight changes studied. The other 2

¹ Patek, A. J., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 329.

² Patek, A. J., and Post, J., *J. Clin. Invest.*, 1941, **20**, 481.

³ Fleming, R. G., and Snell, A. M., *Am. J. Digest. Dis.*, 1942, **9**, 115.

⁴ Gyorgy, P., and Goldblatt, H., *Proc. Soc. Exp. Biol. and Med.*, 1941, **46**, 492.

⁵ Gyorgy, P., and Goldblatt, H., *J. Exp. Med.*, 1939, **70**, 185.

⁶ Lillie, R. D., Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1941, **56**, 1255.

⁷ Daft, F. S., Sebrell, W. H., and Lillie, R. D., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48**, 228.

⁸ Blumberg, H., and McCollum, E. V., *Science*, 1941, **93**, 598.

⁹ Earle, D. P., and Victor, J., *J. Exp. Med.*, 1941, **73**, 161.

¹⁰ Drill, V. A., and Hays, H. W., *Proc. Soc. Exp. Biol. and Med.*, 1941, **43**, 450.

¹¹ Cowgill, G. R., *Am. J. Physiol.*, 1928, **85**, 45.

dogs were maintained throughout the experiment on the modified Cowgill diet, plus yeast, as normal controls. The bromsulphalein method of Rosenthal and White,¹² as previously employed with dogs,¹⁰ was used as a test of liver function.

Results. The liver function of the 2 normal dogs varied from 2 to 12% retention of dye at the end of 30 minutes throughout the experiment. We have regarded any dye retention of 15% or greater at the end of 30 minutes as evidence of an abnormal liver function. Four of the 5 dogs used gave evidence of an abnormal liver function when the yeast was removed from the diet. Dog No. 12 showed an abnormal dye retention on the 27th day of the experiment which increased to 60% retention of dye and then declined to normal. A similar result was obtained with dog No. 13 who developed an abnormal liver function on the 42nd day, which increased to 50% retention of dye and then regressed to normal. Dogs Nos. 25 and 30 first gave evidence of an abnormal dye retention on the 28th and 19th day respectively. One dog, No. 27, did not develop an abnormal liver function during the course of the experiment.

At first it seemed strange that the abnormal liver functions of dogs Nos. 12 and 13, once they had developed, would return to normal instead of becoming progressively worse. However, on studying the food intake of the dogs it was observed that the first evidence of abnormal dye retention was obtained just before, or soon after, the dogs developed complete anorexia. It is possible that the continued anorexia would in turn impose less metabolic work upon the liver, which might then have a greater opportunity to excrete the injected dye.

The removal of the yeast from the diet leads to a decrease in appetite which culminates in anorexia. Himwich, Goldfarb and Cowgill¹³ obtained anorexia in 4 dogs in an average of 32 days. In this experiment the dogs developed anorexia in an average of 35 days, consuming an average of 1,188 calories

per square meter per day before anorexia appeared. Dogs Nos. 13 and 25 developed an abnormal liver function as their appetite was falling, but before complete anorexia ensued. Dogs Nos. 12 and 30 did not develop an abnormal dye retention until after complete anorexia became evident.

On necropsy the gross appearance of the liver was normal in color and size. No mottling or nodularity was present. The liver cut easily and the cut surface appeared normal. The weights of the liver, spleen and kidneys were within the normal range. The heart, however, was hypertrophic, weighing an average of 2 g per kilo of body weight more than normal.

Three different pieces of liver were taken from each dog, fixed in Bouin's solution, and the sections stained with hemotoxylin and eosin. Sections from the liver of dog No. 13 were essentially normal, whereas the livers of the other dogs showed definite pathological changes, varying from a mild to a marked chronic passive congestion. The liver tissue appeared as compact masses with normal nuclei and no evidence of pycnosis. In some sections hyperemia of the sinusoids surrounding the central vein had obliterated the normal hepatic cord appearance. Vacuolization was seen, the cells exhibiting the type swelling and altered cytoplasmic state of cloudy swelling.

Discussion. When the yeast was removed from the diet of the dogs, the increased dye retention did not appear until the food intake had fallen to a low level, or until complete anorexia was present. In this study, therefore, we are dealing with the effect of an acute deficiency of the vitamin B complex, and also with any effect anorexia may have on hepatic function. The results show that as the period of anorexia continued the dye retention returned to normal in 2 dogs. This might tend to show that the lack of food intake as such did not produce the hepatic damage, for if the anorexia was responsible for the abnormal liver function continued anorexia should further increase the liver damage. However, further studies are being made on the effect of anorexia and of a chronic deficiency of the vitamin B complex on liver function.

¹² Rosenthal, S. M., and White, E. C., *J. Am. Med. Assn.*, 1925, **84**, 1112.

¹³ Himwich, H. E., Goldfarb, W., and Cowgill, G. R., *Am. J. Physiol.*, 1932, **99**, 689.

A deficiency of yeast or various B vitamins has been reported to produce histological changes in the liver of rats,^{4,5,14} rabbits,⁷ and guinea pigs.¹⁶ Rhoads and Miller¹⁷ found that there was a lowered capacity of the liver of dogs fed a black-tongue diet to excrete injected bilirubin. The amount of yeast in the diet is also related to the hepatic damage produced by lead arsenate,¹⁸ *p*-dimethylamino-azo-benzol¹⁹ and thyroid feeding.^{10,20} An increased food intake may be a factor in preventing liver damage in such cases.^{21,22}

Conclusions. Five dogs fed a vitamin

complex-free diet showed a gradual loss of appetite which culminated in anorexia. Four of these dogs developed an abnormal liver function, as judged by an increased retention of bromsulphalein. The hepatic damage appeared before, or slightly after, the time that anorexia appeared, and as the period of anorexia continued the dye retention returned to normal in 2 dogs. Histologically, the liver of these dogs showed mainly a mild to a marked chronic passive congestion.

¹⁴ Gyorgy, P., and Goldblatt, H., *J. Exp. Med.* 1942, **75**, 355.

¹⁵ Rich, A. R., and Hamilton, J. D., *Bull. Johns Hopkins Hosp.*, 1940, **66**, 185.

¹⁶ Spellberg, M. A., and Keeton, R. W., *Am. J. Med. Sc.*, 1940, **200**, 688.

¹⁷ Rhoads, C. P., and Miller, D. K., *J. Exp. Med.* 1938, **67**, 463.

¹⁸ Von Glahn, W. C., and Flinn, F. B., *Am. J. Path.*, 1939, **15**, 771.

¹⁹ Sugiura, K., and Rhoads, C. P., *Cancer Research*, 1941, **1**, 3.

²⁰ Drill, V. A., and Hays, H. W., *Am. J. Physiol.*, 1942, **136**, 762.

²¹ Drill, V. A., Shaffer, C. B., and Overman, R., *Am. J. Physiol.*, 1943, **138**, 370.

²² Post, J., Earle, D. P., Patek, A. J., and Victor, J., *Am. J. Path.*, 1942, **17**, 661.

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Ultracentrifugation Studies on the Blood of Normal and Jaundice-diseased Silk worms.

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Through the courtesy of Drs. R. W. Glase and W. M. Stanley, the author was afforded an opportunity to make a limited number of observations with the ultracentrifuge on the blood of normal and of jaundice-diseased silk worms and on an infectious material isolated by high-speed quantity centrifugation from the fluid of diseased worms. Because it is unlikely that it will be possible to extend this study in the near future, it was thought desirable to describe briefly the results obtained thus far.

A Bauer-Pickels type ultracentrifuge^{1,2} equipped with a Svensson-Philpot optical

system^{3,4} was used in this investigation. The results of sedimentation studies on 4 samples of pooled blood from healthy worms are presented in Table I (Samples I, II, III, and IV). It may be observed that 2 major components were found. As judged by the areas under the schlieren diagrams, the amount of material associated with the slower component was from one-half to one-third of the total.

Three of the samples of normal blood listed in Table I were treated by dilution with an 0.18 M sodium chloride-0.02 M sodium phosphate buffer solution at pH 7, by dialysis against an excess of the buffer, or by both. These treated materials, as well as a prepara-

¹ Bauer, J. H., and Pickels, E. G., *J. Exp. Med.*, 1937, **65**, 565.

² Pickels, E. G., *Rev. Sci. Instruments*, 1938, **9**, 358.

³ Svensson, H., *Kolloid-Z.*, 1939, **87**, 181.

⁴ Philpot, J. St. L., *Nature*, 1938, **141**, 283.