36 mm., 6 days; 30 mm., 90 days; 12 mm., 28 days; 10 mm., 22 days; 14 mm., 3 days. Precautions were taken to avoid the errors of observation into which the McGregor method may easily lead when moderate changes in blood pressure are involved. Bilateral denervation, performed in one stage, was quite as well tolerated as unilateral denervation. No changes in the respiratory rates were observed after the rabbits had recovered from the immediate effects of the operation.

To detect any modification in the secretion of epinephrine, the splenic volume, as revealed by the erythrocyte count of the peripheral blood, was utilized. The blood was drawn approximately 15 minutes after completion of the denervation. After unilateral denervation, no changes were observed in the erythrocyte counts. Bilateral denervation in 9 animals was followed by a polycythemia in 5. The maximum increase in the erythrocytes was 1,180,000, the minimum, 420,000. Blood sugar determinations, made after complete recovery of the animals from the operation, were always normal (80 to 105.6).

6902

Effect of Vitamin Deficiency on Gastric Secretion.

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Voegtlin and Myers[1] suggested the identity of vitamin B and secretin, but this could not be confirmed by Anrep and Drummond,[2] Downs and Eddy[3] nor Cowgill and Mendel,[4] who reported that this vitamin exhibits no effect on the liver, salivary or pancreatic secretions. Cowgill, Deuel and Smith[5] state that the restorative action of vitamin B on the appetite is not due to stimulation of gastric secretion, but they made no investigation of the stomach secretions. Miyadere[6] found a great diminution of the secretion from the Pavlov pouch on a vitamin-free diet, yet 100 cc. of 5% alcohol intro-

[1] Voegtlin, C., and Myers, C. N., *J. Pharmacol.*, 1919, **13**, 301.

[2] Anrep, G. V., and Drummond, J. C., *J. Physiol.*, 1921, **54**, 349.

[3] Downs, A. W., and Eddy, N. B., *Am. J. Physiol.*, 1921, **58**, 296.

[4] Cowgill, G. R., and Mendel, L. B., *Am. J. Physiol.*, 1921, **58**, 131.

[5] Cowgill, G. R., Deuel, H. J., and Smith, A. H., *Am. J. Physiol.*, 1925, **73**, 106.

[6] Miyadere, K., *Biochem. Z.*, 1921, **124**, 244.

duced into the main stomach provoked a flow of gastric juice. Farnum[7] reported a diminution in the volume and acidity of the secretion from Pavlov pouches in dogs kept on a beri-beri diet in response to a standard autoclaved meal, to histamine, but not to "gastrin." Her results were somewhat irregular, however, and not quite conclusive. Recently Cowgill[8] quoted Gilman's thesis of 1931 reporting the development of gastric achlorhydria in animals fed on diets lacking vitamin B.

Three dogs with oesophagotomy, gastric fistula and obstructed pylorus were maintained by us in good health for several months with duodenal feedings. One animal had also the pyloric antrum resected. These animals offered an excellent opportunity for the study of vitamin-deficient diets, since ample nourishment could be introduced independently of the capriciousness of the dogs' appetite or its disappearance in vitamin B deficiency. Many abnormalities in animals deprived of vitamin B might be the result of inanition arising from anorexia and not the specific effect of vitamin B deficiency. With the intestinal feedings the amount of food introduced was maintained on the same level during the whole experiment which lasted in each case for more than a month. The loss of weight during this period was insignificant. In one dog the weight varied from 16.1 kilos to 15.8 kilos, in the second from 16 to 15 kilos, while the third dog is still under experiment. None of these animals lost their appetite but eagerly ate all meat offered them in the sham-feeding experiments.

In all 3 animals the results obtained were the same. The experiment consisted of removing all vitamins from the dog's diet. The gastric function was tested by sham-feeding (nervous phase), subcutaneous injections of histamine and the introduction of alcohol into the intestine (chemical phase). The secretion following the introduction of the food into the intestine was also noted. First diminution and then complete cessation of gastric secretion occurred, on which 10 gm. of powdered yeast were added to the diet. In a few days a complete restoration of the secretory function of the gastric mucosa had taken place. An experiment on one animal is quoted below as representative.

One dog was placed on a diet of glucose, casein and olive oil,

[7] Farnum, M. B., *Arch. Int. Med.*, 1926, **37**, 42.

[8] Gilman, A., Dissertation, Yale University, 1931: "Chemical and physiological investigation on canine gastric secretion." Quoted from Cowgill, *J. Am. Med. Assn.*, June 18, 1932.

the vitamins which it had previously received (cod liver oil, yeast and tomato juice) being withheld. Diminution in the volume of the gastric secretion was the first objective evidence of vitamin deficiency. Eight days after the removal of the vitamins, while the animal was in its usual good spirits, 0.75 mg. of histamine injected subcutaneously provoked a secretion of only 4.6 cc. in 30 minutes compared with 56 cc. in the same time when vitamins were included in the diet. Sham-feeding likewise produced only 7 cc. in one hour compared with 76 cc. when the diet was adequate. Ten days later histamine again gave only 3.8 cc. and sham-feeding 2.2 cc. of juice with a large content of mucus in the same periods—this in spite of the previous introduction of 500 cc. of tap water to rule out any possibility of dehydration.

At the end of 3 weeks the animal was somewhat depressed, had liquid stools, but always retained a voracious appetite. 10 gm. of powdered yeast were added to the diet for 3 consecutive days. Tests now showed an almost complete restoration of the secretory function and the animal was once more in good spirits. 0.75 mg. of histamine injected subcutaneously now gave 48.5 cc. of gastric juice and sham-feeding 67.2 cc. in 45 minutes. Under histamine stimulation the free acidity rose to 0.409% and the total to 0.536% HCl, and the peptic power fell to 64 Mett's units. Sham-feeding produced a juice with free acidity from 0.365% to 0.431% and a total of from 0.523 to 0.555% HCl. The digestive power rose to 310 Mett's units.

The yeast was again withdrawn and after 6 days the secretagogue effect of histamine and sham-feeding was again almost abolished, yielding respectively 4.5 cc. and 10.6 cc. At the end of 12 days histamine stimulation produced only 1.0 cc. of thick mucus in 45 minutes.

The following day sham-feeding provoked no secretion whatever and 2 injections of 5% alcohol into the duodenum showed the stomach to be quite refractory apparently to all stimuli. There were only a few drops of thick mucus with no free acidity. The stomach had practically ceased to secrete. The dog soon after this appeared listless and developed a flaccid paralysis of the hind limbs, but retained its appetite. The hair was dry and came out in large quantities. The stools were partly formed, well coloured and occurred only once a day. The blood chemistry was normal, showing CO_2 combining power of 55.2 vol. %, NaCl 550 mg. %, serum calcium 10.6 mg. %, haematocrit reading 40%.

The experiments reported here indicate that the vitamins contained in yeast are necessary for the normal secretory activity of the gastric mucosa. No analyses have yet been made of the B fraction responsible for this effect. The complete achlorhydria under the condition of avitaminosis was obtained independently of anorexia and inanition, which never occurred in our experimental animals.

6903

Cultivation of Louping Ill Virus.

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A simple method[1, 2] for the cultivation of vaccine virus was described which consisted of minced viable chick embryo tissue suspended either in Tyrode's solution or in a mixture of Tyrode's solution and rabbit serum. Recently it has been shown that the viruses of vesicular stomatitis[3] and poliomyelitis[4] are capable of multiplication in media similar to that used for vaccine virus. For the cultivation of the former active agent, minced chick embryo tissue suspended in Tyrode's solution was used, while for the latter minced chick embryo brain tissue suspended in a mixture of monkey serum (1 part) and Tyrode's solution (9 parts) was employed. During the past year we have been investigating louping ill, a neurotropic virus disease of sheep which in some respects resembles poliomyelitis. After the appearance of Gildemeister's note[4] on the cultivation of poliomyelitic virus, it seemed of interest to determine whether another neurotropic virus, the etiological agent of louping ill, is capable of growth *in vitro* under similar conditions.

Two types of media were used. The first (Series A) consisted of 0.1 gm. of minced chick embryo (11 days) brain suspended in a mixture (4.5 cc.) of monkey serum (1 part) and Tyrode's solution (9 parts). The second medium (Series B) was prepared in a manner similar to that of the first with the exception that chick

[1] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

[2] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

[3] Cox, H. R., Syverton, J. T., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 896.

[4] Gildemeister, E., *Cent. Bakt., Abt., 1, Ref.*, 1933, **109**, 284.