found to be sensitive to 0.1 millimicrograms of vitamin B_{12} present in the developed chromatogram. No measureable destruction of vitamin B_{12} was noted in the chromatographic procedure.

In the assay of the liver extract and crystalline vitamin B_{12} all the growth was confined to the first 2 cm section of the chromatograms. The remaining sections of the chromatogram failed to promote any growth response, indicating that the liver extract used in our experiments contained little, or no, thymidine or other growth factors. Interference from thymidine, therefore, did not occur when assaying the liver extract at a level of 0.0045 unit, or less, per tube.

In preliminary work it is necessary to assay each cross sectional piece of filter paper in the entire length of the chromatogram. Once the range of R_f‡ values of any substance has been accurately determined, it is only necessary to test the section known to contain that particular substance. For example, after separating thymidine from vitamin B_{12}, only the first 2 cm section of the chromatogram needs to be tested to obtain the vitamin B_{12} content of the substance being assayed.

The R_f value of vitamin B_{12} was found to be less than 0.04 while the large thymidine zone had an average R_f value of 0.33 and varied between 0.31 and 0.37. This thymidine R_f value differs from that reported by Hotchkiss[8] who obtained an R_f value of 0.51 using the same solvent but in the presence of gaseous ammonia. Winsten and Eigen[2] reported a double zone of growth for thymidine with the principal zone having an R_f value of 0.54 and a minor zone with an R_f value of 0.41. These latter workers were not able to state unequivocally which of these zones was due to thymidine.

The improved procedure has also been used successfully with the ascending solvent technic described by Williams and Kirby.[9] It appears applicable to the study of unidentified growth factors required by other microorganisms as well as *L. leichmannii* (ATCC 4797) and in the study of growth inhibitors and antibiotics.

Summary. A simple, quantitative method for the microbiological assay of filter paper chromatograms has been described. Some possible applications have also been pointed out.

‡R_f (rate of flow) = Distance from starting line to center of growth zone / Distance from starting line to solvent front

[8] Hotchkiss, R. D., *J. Biol. Chem.*, 1948, **175**, 315.

[9] Williams, R. J., and Kirby, H., *Science*, 1948, **107**, 481.

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17196. Duodenal Ulcers Produced on a Diet Deficient in Pantothenic Acid.*

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It is well known that various nutritional essentials are concerned with the maintenance of normal epithelial tissue. As part of a program concerning nutritional deficiencies and gastrointestinal epithelium, we have reported the finding of marked antral gastritis on calcium deficient diets.[1] Similar, less marked and less constant lesions were also reported on thiamine deficiency. Under various conditions of inanition the fundic mucosa will show rather typical areas of hemorrhage.[2] Previously, many papers have appeared on the

* This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

[1] Zucker, T. F., Berg, B. N., and Zucker, L. M., *J. Nutrition*, 1945, **30**, 301.

[2] Zucker, T. F., Berg, B. N., and Zucker, L. M., *J. Nutrition*, 1945, **30**, 319.

involvement of nutritional deficiencies in the production of hyperplasia of the forestomach epithelium of rats.[3] In none of these cases, even when modifications were introduced to accentuate the process, have penetrating lesions been observed which could in the narrower sense of the word be designated as ulcers or which resembled typical ulcers as they occur spontaneously in man.

Further observations have shown that on a diet deficient in pantothenic acid penetrating ulcers are produced in rats. The diet contained all the known nutritional factors necessary for growth except pantothenic acid, and had the following composition per 100 g of diet: vitamin "free" casein (Labco) 18.0; cerelose 71.9; salt mixture 6.1 (Ca 1.0, P 0.55); cottonseed oil 2.0; celluflour 2.0. Incorporated in the oil were 10 mg alpha tocopherol and 1500 international units of vitamin A as carotene. The celluflour carried: thiamine HCl and pyridoxine HCl 1 mg each; riboflavin 2 mg; niacin 4 mg and 2-methylnaphthoquinone 0.5 mg.

Out of a larger group covering various age ranges, 45 animals have been autopsied: 23 older rats with an initial age averaging 394 (271-620) days and 22 younger rats starting at 42 to 105 days. The average experimental time for the older animals was 100 (67-140) days and for the younger ones 125 (87-185) days. As a rule the animals were autopsied when they showed severe weakness or an abrupt loss in weight.

Typical signs of pantothenic acid deficiency consisting of porphyrin deposit on the hair, degeneration, necrosis and hemorrhage in the adrenal cortex and fatty infiltration of the liver appeared in varying degree in all of the rats. Characteristic new findings were atrophy of the duodenal mucosa which was present in every animal, and duodenal ulcers which were encountered in 60% of the rats. The rest of the gastrointestinal tract was unaffected. Several hundred normal control rats and animals kept on diets deficient in other B factors failed to show similar changes in the duodenum.

Areas of atrophy appeared as smooth depressions of various shapes and sizes. Sometimes the greater part of the surface was involved except for a few remaining islands of intact mucosa. Microscopically, villi were absent and were replaced by a flat layer of low cuboidal or squamous-like epithelium. Lesser degrees of atrophy were also noted. Here the villi were shortened or resembled simple tubular glands.

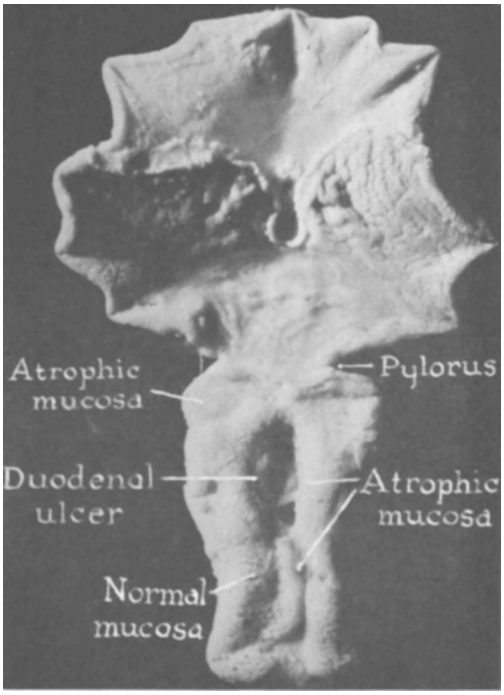

FIG. 1.
Large perforated duodenal ulcer. Base formed by connective tissue and adherent pancreas. Also shown, are areas of mucosal atrophy.

Ulcers have thus far been seen chiefly in atrophic mucosa. They occurred in 17 older rats and in 10 younger ones; in 17 instances multiple ulcers were present. Grossly and microscopically the lesions corresponded to the so-called acute and chronic ulcers of humans. Acute lesions extending for a variable distance through the different layers of the duodenal wall were found in all of the 27 animals. In 6 rats (5 belonging to the older group) coexisting large perforated defects sealed by dense connective tissue and adherent pancreas were also observed. (Fig. 1). A fibrinopurulent peritonitis due to perforation was en-

[3] Berg, B. N., *Am. J. Path.*, 1942, **18**, 49.

countered twice. Hemorrhage from eroded vessels in the fibrous base of ulcers was the cause of death in 2 animals.

In swine, Wintrobe and associates have described atrophy and ulcers in the colon.[4] They also reviewed signs of pantothenic acid deficiency in other species. No duodenal lesions occurred among their reported findings.

Whether the experimentally produced ulcers will acquire significance for the study of human ulcers remains to be seen. The possibility that the latter condition is caused directly by dietary pantothenic acid deficiency is remote considering the exceedingly wide distribution of this factor. To base a working hypothesis on these observations it would be necessary to adduce evidence for abnormal paths of pantothenic acid metabolism. In rats, for instance, with adequate pantothenic acid in the diet but with prevention of intestinal synthesis of folic acid and biotin, a pantothenic acid deficiency in the tissues develops.[5] It is relieved by feeding folic acid and biotin. Orienting studies are under way to test out possibilities of pantothenic acid being involved in the human duodenal ulcer. In the meantime, however, the findings herein reported should be of value in elucidating the various steps of the pathological process which go into the formation of a duodenal ulcer, particularly the early stages which are seldom seen in human material.

[4] Wintrobe, M. M., Follis, R. H., Jr., Alcayaga, R., Paulson, M., and Humphreys, S., *Bull. Johns Hopkins Hosp.*, 1943, **73**, 313.

[5] Wright, L. D., and Welch, A. D., *Science*, 1943, **97**, 426.

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17197. Neutralizing Antibody Against Viruses of the Encephalomyocarditis Group in the Sera of Wild Rats.

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The encephalomyocarditis (EMC),[1] MM,[2] Columbia-SK[3] and Mengo encephalomyelitis[4] viruses have been shown recently to be immunologically indistinguishable,[5,6] hence, they are apparently strains of the same agent. The viruses of the EMC family are now known to have a world-wide distribution, occurring in North America,[1-3] the Philippines,[7] and Africa,[4] but the natural history of the disease and sources of human infection are still obscure.

On at least two occasions viruses of the EMC group have been demonstrated to be pathogenic for man. Neutralizing antibodies against EMC virus have been shown to appear in the convalescent sera of a group of American soldiers who contracted a mild febrile illness colloquially designated as "Three-Day Fever"[7] in Manila in 1945-46. In addition, acute encephalomyelitis caused by Mengo virus occurred in a laboratory worker at Entebbe, Africa.[4]

We were led to consider the possibility that the wild rat might serve as a reservoir for viruses of the EMC type for a number of reasons:

Mice, hamsters and cotton rats are highly susceptible and succumb to even small doses

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[3] Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407.

[4] Dick, G. W. A., Best, A. M., Haddow, A. J., and Smithburn, K. C., *Lancet*, 1948, **2**, 286.

[5] Warren, J., Smadel, J. E., and Russ, S. B., *J. Immunol.*, 1949, in press.

[6] Dick, G. W. A., *J. Immunol.*, 1949, in press.

[7] Smadel, J. E., and Warren, J., *J. Clin. Invest.*, 1947, **26**, 1197.