Increase in resistance to penicillin and loss of virulence have been accompanied by other changes. The rate of growth was retarded and the type of colony became more variable, especially in those cultures showing the greater resistance. Bile solubility of the pneumococcus cultures and type specificity, as determined by the *Quellung* method, were unchanged. Carbohydrate fermentation was the same with resistant and control cultures although it occurred more slowly with the former.

Using the staphylococcus Smith culture, we have been unable to demonstrate acquisition of resistance to penicillin by *in vivo* methods. Twenty serial passages through mice treated with penicillin increased resistance only proportionally to increase in virulence.

Cultures rendered resistant to penicillin were, in our hands, unchanged in their resistance to the sulfonamides, and also to other antibiotic substances, namely, gramicidin, gliotoxin, and aspergillic acid.

Summary. Three cultures of staphylococci, one of *Streptococcus pyogenes* and 3 of pneumococci, Types I, II, and III, showed wide differences in degree of resistance after cultivation in presence of penicillin.

Loss in virulence for mice was associated with increase in resistance. Serial passage of one such strain through mice failed to restore virulence.

Passage *in vivo* in the presence of penicillin failed to increase specific resistance of a strain of *Staphylococcus aureus*.

14173

Calcium Deficiency and Gastric Lesions in the Rat.

THEODORE F. ZUCKER AND BENJAMIN N. BERG.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

Gastric lesions resulting from dietary deficiencies have been described by a number of investigators. As to their cause, almost as many opinions have been expressed. Most of the observations have been made with diets which were deficient in a number of factors.

We have satisfied ourselves that in case of the multiple deficiency diets usually employed the addition of either protein, thiamin, or riboflavin may affect the frequency of lesions or their distribution over the mucosal area. Three histologically different types are found in the 3 anatomically distinguished parts (rumen, antrum, and fundus) of the rat's stomach. Berg[1] has described the histological findings with typical multiple deficiency diets and has given references to previous investigators. The distribution of the lesions is not haphazard, but only partial success has been achieved so far in correlating a single deficiency with a given type of lesion.

In the meantime, it has appeared that another deficiency, namely that of calcium, must be considered. From experiments partly carried out for other purposes, it became apparent that diets adequate for excellent growth in all respects will lead to a high incidence of predominantly antral lesions if calcium is omitted from the salt mixture. These lesions had the same typical appearance as the antral lesions described by Berg: round hyperplastic areas with a central depression representing a defect in the mucosa.

To illustrate this, two series of experiments will be cited. All animals were on the experimental regime for 28 days. Different groups had initial ages of 4, 7, and 10 weeks. The record is presented as the mean count of antrum lesions per rat in each group. The count is based on gross inspection of the stretched out stomach wall. The antral mucosa may contain lymph nodules indistinguishable grossly from the lesions which appear as roundish slightly raised areas with a depressed

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

center. A long series of histological controls has shown that lymph nodules are not likely to average as high as one per rat even in small groups. In 2 out of over a hundred instances as many as 3 nodules were found in a single rat, while about half of the animals showed no nodules. A count of less than one therefore stands for a normal antral mucosa.

The basal diet of Series 1 contained: Heated egg albumen, 20%; modified Wesson salt mixture, 1.2%; cottonseed oil (containing carotin), 2%; rice bran extract, 5%; P (as KH_2PO_4), 0.4%; cane sugar to make 100%. This was the experimental diet in Series 1. The control diet had 0.6% of Ca added as $CaCO_3$.

Omitting the account of intermediate levels which gave intermediate results, the first series comprised 70 low Ca animals and 65 controls. In groups of 4 or 5 animals of each sex, they were spread over the time from June, 1941, to October, 1942, with no noticeable differences in the summer and winter months. On certain other diets, such as those mentioned by Berg, very marked seasonal differences are obvious.

The groups of 8 to 10 animals showed a mean count of 10 to 18 lesions per rat, averaging 15 for the total of 70 animals. A small number of fundic and rumen lesions were a constant accompaniment of antral lesions on such diets, while with many multiple deficiency diets all types occurred freely. Not infrequently the same stomach showed marked lesions in rumen, antrum, and fundus.

The mean count for the 65 control stomachs was 0.83 per rat with no group being responsible for more than the average except one which contained a rat with 16 lesions. This sporadic occurrence of numerous gastric lesions in normal control groups has also been encountered in other series of experiments. Stock diet animals also show abnormal gastric mucosæ to the extent of about one in a hundred.

Growth and food intake on Ca deficient diets may remain approximately normal for 7 to 10 days. After this, both fall off sharply. The protein and B complex allowances in the diets mentioned above are quite adequate with normal food intakes. It might be held that in such an experiment the effective cause need not be Ca directly, but rather a deficiency induced by lowered intakes of other factors. The second series aimed at minimizing the chances of such an occurrence.

In the second series, the protein was raised to 26.7%. The rice bran extract which as a source of B complex is relatively deficient in riboflavin was supplemented by adding 100 γ of riboflavin per 100 g of diet. In Series 1, the egg albumen supplied considerable riboflavin. An additional source of B complex (Harris yeast extract) was used to insure against borderline values of the less well worked out or unidentified B factors. The phosphate added to the diet was adjusted for the P content of the casein. The constituents common to the 4 diets used were as follows: Casein, 26.7%; cottonseed oil and carotin, 2.0%; modified Wesson salts, 1.2%; KH_2PO_4, 0.66%; rice bran extract, 5%; riboflavin, 100 γ; cerelose to 100%. The variables and the antrum lesion counts are given below:

	Diet 1015	Diet 1016	Diet 1018	Diet 1017
Yeast extract	0.5%	0.5%	2.0%	2.0%
$CaCO_3$	1.49%	0	1.49%	0
No. of rats	8	9	6	15
Lesion count	0	24	0	18

Six rats on diet 1018 were pair-fed to 6 rats on 1017. With the food intake on the adequate diet reduced (maximally about 50%) to that of the calcium deficient one, the gastric mucosa remained perfectly normal. While in the experiments of Series 1 very few lesions in either rumen or fundus were seen, Series 2 showed none. The lesions were exclusively antral.

Discussion. A comparison of Series 1 and 2 appears to give more information than merely corroborating the indication that it is a calcium deficiency *per se* which produces the result. Since on no other diets except those of Series 2 have we ever obtained antrum lesions uncomplicated by fundus or rumen lesions, the conclusion seems warranted that reduced food intake can induce complications unless secondary deficiencies are guarded against.

From the experiments given here it is clear that vitamin D deficiency plays no primary role in causation of the lesions. With no other change in the diet, marked lesions or complete

prevention are the result of omission of calcium from or addition of calcium to the diet, respectively. Therefore, since there is complete prevention, in the absence of vitamin D, calcium deficiency *per se* must be regarded as the causative factor. The situation is analogous to that of phosphorus deficiency in experimental rat rickets (Zucker *et al.*,[2] pp. 147-8). Vitamin D cannot fully compensate for very low supplies of the inorganic essential substances (calcium or phosphorus). Only with moderate deficiencies is it possible for vitamin D to improve the net absorption sufficiently to effect prevention. The preventive role of D in gastric lesions produced by moderate calcium deficiency together with other findings in calcium deficiency will be discussed in another report.

Preliminary experiments indicate that the results of Schiödt,[3] who apparently produced exclusively fundus lesions, can be duplicated. No one else has reported such results and we have not seen them until recently. Here the deficiency seems to be one of the B factors as indicated by Schiödt. Further details are now under investigation.

Conclusion. Antral gastric lesions in the rat are produced by calcium deficiency.

2 Zucker, T. F., Hall, L., and Young, M., *J. Nutrition*, 1941, **22**, 139.

3 Schiödt, E., *Acta Med. Scand.*, 1934-35, **84**, 456.

14174

Complement Fixation Test with Sera of Animals Immunized with Rabies Virus.

H. BERNKOPF AND D. NACHTIGAL. (Introduced by I. J. Kligler.)

From the Department of Hygiene and Bacteriology, Hebrew University, Jerusalem.

Recently, a reliable technic for complement fixation for diagnosis of rabies was devised by Casals and Palacios.[1] The procedure for preparing antigen is, however, complicated and it seemed desirable to develop a simpler technic for complement fixation which might find application in routine laboratory diagnosis. At first, specific and sharp results were obtained by following the technic developed by Howitt;[2] subsequent experiments showed, however, that further simplification was possible and the following technic was finally evolved, which offers a practical procedure for routine diagnosis.

Antigen. One to 2 g of rabies-infected brain are minced in a Petri dish, spread in a thin layer, and dried in a desiccator over sulphuric acid, at 37°C for 24 hours.* The dried material is scraped off and stored in rubber-stoppered tubes in the ice box. Material thus preserved keeps at least 9 months. For the preparation of antigen, the dried material is ground in a mortar, taken up in neutral physiological saline and added gradually to give a final concentration of 2%. The suspension is centrifuged for 15 minutes at 3000 r.p.m. The supernatant fluid serves as the antigen. This type of antigen can be prepared from the brains of rabbits, guinea pigs, mice, and dogs, infected intracerebrally or peripherally with mouse passage fixed virus, virus from tissue cultures,[3,4] egg passage virus,[5] or with a local strain of street virus. Antigens prepared from brains of guinea pigs and mice infected with equine encephalitis

1 Casals, J., and Palacios, R., *Science*, 1941, **93**, 162; *J. Exp. Med.*, 1941, **74**, 409.

2 Howitt, B. F., *J. Immunol.*, 1937, **33**, 235.

* The preliminary drying is important. Antigens prepared from fresh undried material were found anticomplementary.

3 Webster, L. T., and Clow, A. D., *Science*, 1936, **84**, 487; *J. Exp. Med.*, 1937, **66**, 125.

4 Bernkopf, H., and Kligler, I. J., *Brit. J. Exp. Path.*, 1937, **18**, 481.

5 Kligler, I. J., and Bernkopf, H., *Nature*, 1939, **143**, 899; Bernkopf, H., and Kligler, I. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 332.