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Induced Resistance to Penicillin of Cultures of Staphylococci, Pneumococci and Streptococci.

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It has been repeatedly shown that various infectious agents on exposure to certain substances acquire resistance to those substances. Abraham, Chain and collaborators¹ have rendered a culture of *Staphylococcus aureus* resistant to penicillin by growing it in broth containing increasing concentrations of penicillin. Rammelkamp and Maxon² have reported the development of resistance *in vitro* to penicillin by two strains of staphylococci. They also reported the recovery of resistant strains from 4 out of 14 patients with staphylococcal infections which were treated with penicillin.

It seemed of interest to determine if other organisms could likewise be rendered resistant to penicillin. Three strains of pneumococci, Types I, II, and III, one strain of *Streptococcus pyogenes*, C-203, and 3 strains of staphylococci were passed serially in broth containing the greatest amount of penicillin which would permit growth. Control cultures were passed in the same broth without penicillin. Subcultures were made at frequent (usually daily) intervals. Fuller details of this work will be reported elsewhere.³ The development of resistance was shown by the ability of the cultures to grow in increasing concentrations of penicillin. At intervals comparative tests of the resistance of control and penicillin-passed cultures were made. The passages in penicillin broth extended over a period of 3 months and with all but 2 of the cultures 56 to 60 passages were made. These 2, both staphylococcus cultures, had only 28

passages each and acquired a resistance respectively 1000 and 4000 times greater than that of the control cultures. The third staphylococcus culture which had 60 passages acquired a 6000-fold increase in resistance. The Type III pneumococcus and the *Streptococcus pyogenes* acquired a 30-fold increase in resistance, while the Type I and Type II pneumococci showed only a 6-fold increase.

Cultures which have become resistant to penicillin, unlike those resistant to sulfonamides, show marked decrease in virulence for mice. Five of the 7 cultures passed in penicillin broth lost virulence to a marked degree, the 2 which showed only slight loss were the Type I and Type II pneumococcus cultures which had acquired only 6-fold resistance *in vitro*. The other cultures, with increase in resistance varying from 30- to 6000-fold, showed equal and almost complete loss in virulence. Cultures which, before passage, killed regularly in a dilution of 10^{-7} after passage killed only irregularly in a dilution of 10^{-1} . This loss of virulence, once acquired, appears to be a permanent characteristic of the organism. The Type III pneumococcus resistant culture was subjected to 9 serial passages in mice and 32 rapid transfers in blood broth. Neither procedure increased virulence or decreased resistance to penicillin *in vitro*. After passages in penicillin broth were stopped all the cultures were stored in the icebox with only occasional transfers to fresh tubes of broth. After 2 months storage there was no change in either resistance *in vitro* or virulence. The 2 least resistant staphylococcus cultures were not tested. It was noticed, however, that the penicillin resistant strains grew more quickly on transfer to fresh broth, and colonies on blood agar grew more luxuriantly than at the time passage in penicillin broth was stopped.

¹ Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **2**, 177.

² Rammelkamp, D. H., and Maxon, T., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51**, 95.

³ Rake, G., McKee, C. M., Hamre, D. M., and Houck, C. L., to be published.

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*.

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Calcium Deficiency and Gastric Lesions in the Rat.

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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. Berg¹ 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.