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The Biologic Nature of Sulfapyridine's Bacteriostatic Effect Against the Pneumococcus.

RALPH R. MELLON AND RUTH A. MCKINNEY.

From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh.

This paper represents an experimental attempt to show that the morphologic alterations characteristically induced by the sulfonamide compounds against the pneumococcus are of the nature of a phasic dissociative change rather than "involutionary", in the monomorphic sense. This in accordance with our position as previously outlined at the June, 1939, meeting in Milwaukee of the American Association for the Advancement of Science. (In press.)

Two series of mice were employed: the first of 20 animals, and the second of 10, with 10 controls for each. The animals were infected intraäbdominally with 0.5×10^{-6} cc of a 16-hour veal-infusion broth-culture of Type II pneumococcus (Binda strain). Therapeusis was started in 2 hours and consisted of 4 doses daily at 4-hour intervals, of 0.25 to 0.5 cc of a 1:4 dilution of a sulfonamide carbohydrate compound.* Cultures and smears of peritoneal exudate were made at alternate treatments between the 3d and 15th.

After 16 hours the first response was obtained. It consisted of a marked clumping of the organisms both about the cellular elements and independently. After 20 hours, the free organisms in the smears showed capsules so swollen as to resemble a Neufeld reaction. There was also a definite phagocytosis of the swollen organisms by the polynuclear and macrophagic cells, whose margins were no longer clear-cut. Adherent to them were microörganisms and a granular material, possibly fibrin.

At this time the mucoid colonies were slightly umbilicated and microscopically contained a pleomorphic phase that, while not isolatable at this time, at the 40-hour period dissociated as a dwarf colony of non-encapsulated pneumococci reacting negatively to the Neufeld-test. It was bile-soluble, fermented inulin slowly, and morphologically the cocci were elongated to a point where they often resembled diphtheroids; or they formed long chains. Their methemoglobin-

* In order to increase its solubility the sulfapyridine is dissolved in galactose and nucleic acid. To this mixture is added some sulfanilamide that has been combined with xylose, which completes the solubility of the sulfapyridine. Dr. Fritz Meyer and his associates of New York are responsible for this preparation and we are indebted to him for the samples used in this study.

formation was greatly diminished. The "dwarf" strain was further dissociated into a minute or G-type colony (Hadley) whose morphology was that of a micrococcus instead of a diplococcus. Both the "dwarf" colony and the G-type were avirulent.

After the 7th and 9th treatments phagocytosis of the encapsulated organisms markedly increased and the number of free organisms diminished. Their clumping, both free and about the cells, was still evident. After the 11th treatment there was suggestive evidence of lysis of the organisms. This was more marked after the 15th treatment when the cultures were sterile.

The morphology of the encapsulated pleomorphic forms in the umbilicated mucoid colonies is so similar to that of the Neufeld-negative dwarf colonies that we regard the latter as the direct descendants of this pleomorphic phase. In fact, the latter which was diphtheroid-like in morphology, has now been isolated as an independent entity. Moreover, the pleomorphic forms and diplococci, both with swollen capsules, are apparently phagocytable even before their colonial dissociation as a phasic entity occurs. The relative ease with which Neufeld-negative colonies revert to the virulent mucoid phase,[1] together with their other characters, suggests that they fall into a different category of variational change from the M, S, and R pattern; although the fact that Hadley has recently shown that similar changes with the hemolytic streptococcus *in vitro* can carry through to a typical S-colony form, makes it appear that the two categories are essentially the same in nature. In other words, the pleomorphic diphtheroidal forms of the mucoid culture, at a time when they are not yet dissociable, have a *true phasic status* by virtue first, of their phagocytability, and second by a diminution of their principal metabolic activities, such as methemoglobin formation and bile solubility.

The early *in vivo* phagocytability of these pleomorphic phases which thus presages their early destruction, probably explains the clinical and experimental infrequency[1, 2] of the Neufeld-negative or other non-virulent dissociant such as the S-colony. We are inclined to regard the capsular change merely as a part of the dissociative picture rather than a specific effect of the drug on this structure *per se*.

It would appear then, that the bacteriostatic action of the drug *in vivo* is essentially of the nature of a phase-transformation, which brings the germ within killing range of the immuno-phagocytic defenses of the host. This point of view is in accord with the necessity for the administration of repeated small doses, whose effect both *in vivo* and *in vitro* is dominantly dissociational rather than bactericidal.

1 Hilles, C., and Schmidt, L. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 73.
2 Telling, M., and Oliver, W. A., *Lancet*, 1938, 234.

These phasic changes are regarded as being implemented chiefly, but not necessarily solely, by the accumulation of hydrogen peroxide, which may be accounted for on the basis of our demonstration of the anticatalase-action of the sulfonamide compounds.[3-6]

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Choline Esterase Activity in Blood Serum and Duodenum of Beriberi Pigeons.

WILLIAM ANTOPOL, SUSI GLAUBACH AND DAVID GLICK.
From the Division of Laboratories, the Newark Beth Israel Hospital, Newark, N. J.

It has been recently demonstrated by Glick and Antopol[1] and by Süllmann and Birkhäuser[2] that thiamine exerts an inhibitory action upon choline esterase *in vitro*. Zeller, Schär and Staehlin[3] showed that the splitting of histamine and diamines by the diamino-oxidase is also inhibited by thiamine. It has been shown by Goodhart and Sinclair[4] that cocarboxylase is contained in the cellular elements of the blood and the free thiamine essentially in the plasma. In contrast to thiamine, cocarboxylase recently has been found to have very little inhibitory effect upon the enzymes.[5] The question then arises whether thiamine has a physiological effect, *in vivo*, on choline esterase. To throw light on this point measurements were made of the activity of the enzyme in the serum of beriberi and normal pigeons. The concentration of the enzyme in the small intestine was also measured and correlated with its sensitivity to acetylcholine.

Twenty-two pigeons in 3 series were used in these investigations. In the first group, 6 pigeons were placed on a polished rice diet and choline esterase determinations were made about every 8 days on blood serum obtained from the wing vein. When the pigeon showed

3 Locke, A., Main, E., and Mellon, R. R., *Science*, 1938, **88**, 620.

4 Main, E., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

5 Shinn, L. E., Main, E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 591.

6 Shinn, L. E., Main, E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 640.

1 Glick, D., and Antopol, W., *J. Pharm. Exp. Therap.*, 1939, **65**, 389.

2 Süllmann, H., and Birkhäuser, H., *Schweiz. Med. Wchnschr.*, 1939, **69**, 688.

3 Zeller, E. A., Schär, B., and Staehlin, S., *Helv. Chim. Acta*, 1939, **22**, 837.

4 Goodhart, R. S., and Sinclair, H. M., *Biochem. J.*, 1939, **33**, 1099.

5 Glick, D., and Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 396.