CARBONIC ANHYDRASE INACTIVATION AS THE SOURCE OF SULFANILAMIDE "ACIDOSIS"

SULFANILAMIDE produces its desirable bacteriostatic effects and the greater part of its undesirable disturbing effects on acid-base balance,[1] respiration and circulation, through two distinct types of action. The one is exerted through the p-amino group and the other through the opposed, sulfonamide group. Both actions are anti-enzymatic. The one is directed, principally, against catalase and the other against carbonic anhydrase.

The evidence for the anticatalase property of sulfanilamide has already been presented.[2] The evidence for its anti-carbonic anhydrase property is brought forward in the following paragraphs and in a communication by Mann and Keilin[3] which came to our attention as this note was in preparation and which corroborates, in every essential detail, the independently obtained findings reported herewith.

The investigation was suggested by the observation that the degree of tolerance for inhaled CO_2 can be used as a gauge for judging "fitness."[4] Persons optimally fit, as indicated by a minimum incidence of common cold, had been found to make a significantly less excessive respiratory response to inhaled CO_2 than persons with impaired fitness. The fit person appeared to be able to dilute inhaled CO_2 and convert it into non-stimulating[5] bicarbonate more rapidly than the person less fit. The obvious factors of circulatory efficiency, blood volume and CO_2-combining power were considered and attention turned to carbonic anhydrase. This enzyme facilitates, as one of its functions, the conversion of metabolic (or inhaled) CO_2 into bicarbonate.[6]

Markedly decreased tolerance for inhaled CO_2 had been observed during sulfanilamide administration. An arthritic, receiving daily inhalations of 10 per cent. CO_2 in O_2,[7] was able to tolerate 30 liters without difficulty, before beginning and after concluding sulfanilamide therapy. During the therapy he was able to endure only 7.5 liters.

Test was made of the carbonic anhydrase inactivating power of sulfanilamide *in vitro*. A colorimetric method devised by Philpot and Philpot[8] was used in preference to the manometric method used by Mann and Keilin. The colorimetric method measures the time required for conversion of CO_2 into bicarbonate, instead of the converse decomposition of bicarbonate into CO_2. It is significant that both methods led to identical result. In the absence of carbonic anhydrase the average time required for the indicator change marking the endpoint as described by Philpot and Philpot was 68 seconds, while in the presence of 0.0025 cc of laked, defibrinated rabbit blood, 33 seconds was required. The substances tested for inhibitory power were dissolved in the CO_2 solution or added as alcoholic solutions to the reaction mixture in amounts sufficient to give final concentrations of 0.0005 M. Catalysis was completely inhibited by sulfanilamide, acetylaminobenzenesulfonamide, p-toluenesulfonamide and p-caproylaminobenzenesulfonhydroxamide. Sulfapyridine, sulfathiazole, methyl-p-aminophenylsulfone and p-aminobenzoic acid had no effect. The tested compounds were differentiated in their actions against a partially purified preparation of carbonic anhydrase exactly as was found against diluted blood.

Inhibition was apparent only with compounds containing an unsubstituted sulfonamide or sulfonhydroxamide group. It was in no way dependent on possession of the p-amino group known to be essential for therapeutic activity.

Sulfapyridine and sulfathiazole, producing no inactivation of carbonic anhydrase *in vitro,* produce no effects comparable to those produced by sulfanilamide on acid-base balance *in vivo*.[9] The effects produced by sulfanilamide are immediate,[10] requiring for induction only the time necessary for absorption. Both sulfanilamide and sulfocyanate (first shown to be an inhibitor of carbonic anhydrase, *in vitro* and *in vivo,* by Davenport[11]), when given to rabbits in amounts sufficient to give concentrations in the blood equal to or higher than those effecting carbonic anhydrase inactivation *in vitro,* cause the respiratory distress and lengthening of the warming time[12] which would be anticipated to follow carbonic anhydrase inactivation *in vivo*. Loss of appetite is induced, possibly because of a lessened capacity for secreting gastric acid, which can be produced in optimum quantity only when the

[1] A. F. Hartmann, A. M. Perley and H. L. Barnett, *Jour. Clin. Invest.*, 17: 465, 1938.

[2] A. Locke, E. R. Main and R. R. Mellon, SCIENCE, 88: 620, 1938; *Jour. Immunol.*, 36: 183, 1939; E. R. Main, L. E. Shinn and R. R. Mellon, *Proc. Soc. Exp. Biol. and Med.*, 39: 272, 1938.

[3] T. Mann and D. Keilin, *Nature,* 146: 164, 1940.

[4] A. Locke, *Jour. Immunol.*, 39, 1940.

[5] O. Bang, O. Boje and M. Nielson, *Skand. Arch. Physiol.*, Suppl., 10: 1, 1936.

[6] N. U. Meldrum and F. J. W. Roughton, *Jour. Physiol.*, 80: 113, 1933.

[7] A. Locke and M. A. Cohen, *Proc. Soc. Exp. Biol. and Med.*, 43: 611, 1940.

[8] F. J. Philpot and J. St. L. Philpot, *Biochem. Jour.*, 30: 2190, 1936.

[9] P. H. Long, J. W. Haviland, L. B. Edwards and E. A. Bliss, *Jour. Am. Med. Assn.*, 115: 364, 1940.

[10] W. W. Beckman, E. C. Rossmeisl, R. B. Pettingill and W. Bauer, *Jour. Clin. Invest.*, 19: 635, 1940.

[11] H. W. Davenport, *Am. Jour. Physiol.*, 129: 505, 1940.

[12] A. Locke, *Jour. Immunol.*, 36: 159, 1939.

carbonic anhydrase of the parietal cells can function unrestricted by inactivating agents.[13] Carbonic anhydrase injection, into dogs, is reported to produce a rise in alveolar CO_2,[14] indicating increased tolerance.

Fuller, Colebrook and Maxted[15] find that the growth of most group A hemolytic streptococci in human blood is favored by increase in CO_2 and retarded by decrease. In the blood stream, inactivation of carbonic anhydrase would tend to retard conversion of metabolically produced CO_2 into bicarbonate—an action possibly favoring growth and counteracting bacteriostasis. Bicarbonate is reported by Pappenheimer and Hottle[16] to be no substitute for CO_2 for the maintenance of optimum growth. The possession by sulfanilamide of an unmodified sulfonamide grouping capable of producing inactivation of carbonic anhydrase may, therefore, be a source not only of increased toxicity but also of lowered bacteriostatic effectiveness.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

REUNION OF STUMPS OF SMALL NERVES BY TUBULATION INSTEAD OF SUTURE[1]

IN the course of extensive experiments with peripheral nerves of amphibians,[2] recently extended to rats,[3] a method of nerve union has been perfected which, owing to its adequacy and wide applicability, deserves to be placed on record. The problem is to appose closely the cut surface of a proximal nerve stump, as the source of regenerating fibers, to the cut surface of a distal stump, as the channel into which the fibers are to be routed. Apposition by ordinary suturing can never be precise enough to prevent masses of fibers from escaping into the surroundings and straying off to uncontrollable destinations. Moreover, when we are dealing with nerves of only a fraction of a millimeter in diameter, neat suturing becomes a mechanical impossibility. Both difficulties can be met by tubulating the nerve ends with a tightly fitting cuff of fresh artery.[4]

A fragment of artery slightly narrower than the width of the nerves to be united is chosen, squeezed free of blood and immersed in Ringer's solution. All further manipulations take place in this solution. For instruments we use two pairs of hard steel (watchmaker's) forceps ground down until the ends have become very slender and sharp-pointed. The steps of the operation are illustrated in the accompanying figures. (1) With forceps F pull artery A over closed forceps G; artery becomes greatly dilated. (2) Open forceps G slightly and grasp perineurium of nerve stump NP. (3) With forceps F strip artery from G and pull half-way over NP. (4) Withdraw G. (5) Insert F into empty end of artery and open prongs slightly; introduce nerve stump ND into the opening, until the two stumps meet (some additional

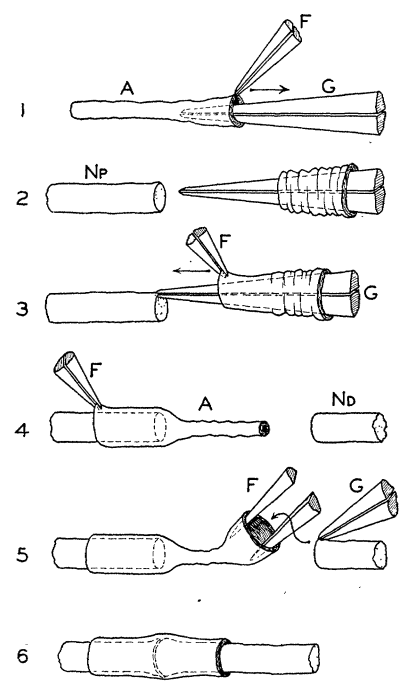

FIG. 1

pressure, flanging the ends, is advisable). (6) Stretch the arterial cuff until it fits snugly.

Enough slack should be allowed to the nerve stumps to insure apposition without stress. After sucking and blotting the excess Ringer's solution from the wound, clotting occurs rapidly. The arterial cuff, firstly, provides a firm link between the nerve ends and, secondly, prevents the formation of a neuroma

[13] Davenport, *op. cit.*
[14] F. Schmitt, *Deut. Arch. klin. Med.*, 134: 300, 1939.
[15] A. T. Fuller, L. Colebrook and W. R. Maxted, *Jour. Path. Bact.*, 48: 443, 1939.
[1] Research aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.
[2] Paul Weiss, *Biol. Rev.*, 11: 494, 1936.
[3] R. W. Sperry, *Jour. Comp. Neurol.*, 73: 1940.
[4] Tubes filled with various media have been used by surgeons to bridge nerve defects. In the present note tubulation is introduced in a different capacity and on a different order of magnitude.
[16] A. M. Pappenheimer, Jr. and G. A. Hottle, *Proc. Soc. Exp. Biol. and Med.*, 44: 645, 1940.