SUCCESSION OF COLI-AEROGENES ORGANISMS IN THE HEALTHY ADULT FECAL FLORA

WE[1] have called attention to the succession of coli-aerogenes forms which takes place when normal human feces are suspended in saline and held at 37° C. and in the ice box. Storage effects a complete turnover in the flora of this group, the predominant Escherichia (coli group) of fresh feces giving way, stage by stage, to an ultimately lactose-degraded Aerobacter (aerogenes-cloacae group) flora in a few weeks time.[2] Exceptions to this generalization occur whenever the original freshly excreted specimen contains (as far as this bacterial group is concerned) only coli. Such exceptions, which are not rare, lend support to the contention that the changes in flora observed represent succession rather than variation.

Further work reveals that the coli-aerogenes flora of fresh feces from healthy adult humans may present such deviation from the accepted normal that it is probable this group maintains its recognized distribution by a balance so delicate that it may occasionally be much disturbed or the organisms even temporarily eliminated. The coli-aerogenes group in fresh feces is ordinarily represented by more than 90 per cent. of coli and less than 10 per cent. of aerogenes with an occasional coli-aerogenes intermediate (Citrobacter) appearing. Frequently specimens are encountered from which only coli can be grown. On the other hand, samples are met with in which the flora of this group is largely or entirely citrate utilizing (Aerobacter and/or Citrobacter). And there are those from which no organisms of the group may be isolated either by direct plating or by plating after preliminary enrichment in lactose broth.

Illustrative cases will make the point clear and point to its significance. Fifty-seven fresh fecal specimens deriving from "A" at intervals between March 21, 1933, and April 23, 1935, were examined and 661 strains carefully studied. In addition several hundred other strains were studied only for their ability to utilize citrate as their sole carbon source. Direct platings were made on Endo's medium from the fecal suspensions and lactose broth enrichment tubes seeded. These last were only utilized in case no growth took place on Endo's medium. Strains for the complete study were after careful purification tested for their dissimilation of dextrose, lactose, saccharose, dulcite, salicin, alpha-methyl-d-glucoside and cellobiose; for the production of acetyl-methyl-carbinol, indol and hydrogen sulfide; for their action on milk and gelatin, for their methyl red reaction; and for their ability to utilize citrate as the sole carbon source. The coli-aerogenes flora of this subject was on 51 occasions predominatingly coli (96.3 per cent.).

However, from his specimen 45 of December 28, 1934, no organisms of the group could be isolated, even utilizing enrichment methods. Five days later a sample showed only citrate utilizing, hydrogen-sulfide producing coli-aerogenes intermediates (50 studied). This is a form rare in the usual flora. On January 7, 1935, specimen 47 was analyzed. Of 20 colonies studied, 17 were aerogenes and intermediates, 3 coli. Two days later a sample yielded no organisms of the group on adequate direct plating. By enrichment procedures coli was obtained. Three days later no organisms could be obtained directly from specimen 49, but by enrichment aerogenes and intermediates were recovered. On January 13, 1935, the fiftieth specimen examined for this subject again yielded no group organisms on direct plating but a sparse yield of coli was obtained via enrichment. Two days later and on six subsequent occasions specimens were analyzed and at each analysis organisms were readily obtained by direct plating. Seventy of 72 strains intensively studied were coli and of 161 additional strains tested on citrate only all failed to develop (coli).

Again, the "C. Y." specimen of February 6, 1935, yielded 29 citrate utilizers of 52 strains examined, but his specimen of February 28 was 100 per cent. coli—35 colonies fished and studied. The "D. K." sample of March 25, 1935, which yielded 80 per cent. citrate utilizing coli-aerogenes, was followed in our series by a 100 per cent. non-citrate utilizing flora for the specimen of April 13. Lastly, "E. B. C." submitted fecal specimens on March 5 and 15, 1935. From neither could coli-aerogenes organisms be isolated, even by enrichment procedures. The third specimen, submitted on April 23, was, however, entirely coli—62 colonies studied. All subjects were in good health and on average mixed diet with the possible exception of "E. B. C.," who was dieting. It should be noted that this diet, to avoid weight, was no more rigorous than very many of our population subject themselves to and in view of the prevalence of such procedures if the diet had anything to do with the results obtained it only makes the point more significant.

The importance of these findings, chosen from 100 rather complete analyses of fresh fecal specimens from 31 different persons, should be apparent. It is possible that in any contamination of water or food stuffs from community excreta the generally accepted coli aerogenes picture may be valid. When, however, one considers contamination from a single source it is

[1] L. W. Parr, *Proc. Soc. Exp. Biol. and Med.*, 32: 580, 1935.

[2] L. W. Parr, *Proc. Soc. Exp. Biol. and Med.*, 31: 1019, 1934.

entirely possible for gross fecal pollution to be present in the absence of recoverable colon bacilli or even of any members of the coli-aerogenes group. This fact may explain certain explosive outbreaks of water-borne disease, such as have occasionally been reported —outbreaks with gastro-intestinal symptomatology and probable etiology but unsolved as to microbial causation. These data explain discrepancies in existing data on the coli-aerogenes distribution in normal feces. Changes in flora of the type reported have been noted, though rarely, in pathological conditions. That such change may occur in normal subjects has not been emphasized and renders less significant such reports in cases of disease. These findings make it increasingly clear that there is no possibility, as yet, of distinguishing between fecal and non-fecal forms of the coli-aerogenes group in any specific instance, despite the probability that in general non-citrate utilizing coli are typically fecal and the other forms of the group less so. If a presumably healthy individual may for days pass feces from which the bacillus nominated as the fecal indicator par excellence can not be recovered one may ask whether there is not still work to do in sanitary bacteriology. If the coli-aerogenes group is our best indicator of intestinal contamination the need for rigid control of water and food processing is emphasized from a new angle. Not the least interesting of the points raised is the question of the mechanism whereby the lactose-fermenting, gram-negative, non-sporing aerobes of the bowel are so grossly disturbed in their relationships or indeed temporarily eliminated in the apparently healthy adult. Bacterial antagonisms and inhibitions, bacteriophage activity, and sub-clinical infections involving disturbances of bowel conditions are a few only of the points which may well be looked into in this connection.

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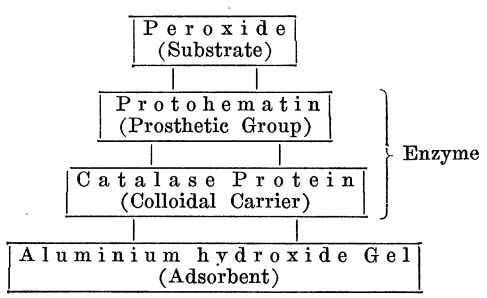
MODE OF COMBINATION OF AN ENZYME WITH AN ADSORBENT AND WITH A SUBSTRATE

IF a purified and highly active solution of liver catalase[1] is adjusted to pH 5 and stirred with a suitable amount of aluminium hydroxide gel or of silicic acid, a large fraction of the enzyme is deposited on the adsorbent. The adsorbate is washed with and suspended in distilled water. When placed in an optical trough of 1 cm thickness and examined in the condensed beam of a 500 watt projection lamp by means of a pocket spectroscope, a three-banded absorption spectrum is observed which proves to be exactly the same as that of the enzyme in solution.[2] The adsorbate is catalytically active towards hydrogen peroxide and monoethyl hydrogen peroxide. With the latter substrate, the two-banded spectrum of the intermediate enzyme-substrate compound and the whole reversible spectral cycle, as reported for the dissolved enzyme,[3] may be seen. Likewise, cyanide will combine with the hematin grouping of the enzyme in the adsorbed state.

Inasmuch as it is known that invertase is eluted from an adsorbate by its substrates, it was necessary to ascertain whether the catalytic activity of the adsorbate in the present case is not due to a preceding desorption of catalase by the peroxide. However, when an adsorbate suspension to which an excess of monoethyl hydrogen peroxide had been added was subjected to filtration while the enzymatic reaction was in progress, a colorless, catalase-free filtrate was obtained, proving that the catalysis and the spectral changes occur directly on the surface of the adsorbate.

The extent of adsorption of the enzyme depends on the type of adsorbent used. Kaolin, finely powdered quartz and activated charcoal did not give satisfactory results under similar experimental conditions.

The enzyme catalase consists of protohematin[4] and of a specific protein.[5] During the heterogeneous catalysis the following arrangement exists:



Free hematin or pyridine-parahematin will not form compounds with peroxides of the type obtained in the case of catalase or methemoglobin. Considering the much smaller activity of methemoglobin compared with that of the enzyme, it follows that not only is a protein component apparently necessary for the formation of the intermediate compound but also the specific nature of the protein determines the decomposition rate of the intermediate. The protein may provide for proper spacing of the hematin groups on the enzyme surface.

It is suggestive to depict the enzyme-substrate compound in the manner preferred by Haurowitz[6] for the

[1] Prepared according to Zeile and Hellstroem.[2] The monomolecular velocity constant of the solution used for these experiments was k = 4525.

[2] K. Zeile and H. Hellstroem, *Z. physiol. Chem.*, 192: 171, 1930.

[3] K. G. Stern, *Nature*, 136: 335, 1935.

[4] K. G. Stern, *Nature*, 136: 302, 1935. *J. Biol. Chem.*, 112: 661, 1936.

[5] K. G. Stern, *Z. physiol. Chem.*, 208: 86, 1932; 217: 237, 1933.

[6] F. Haurowitz, *Z. physiol. Chem.*, 232: 159, 1935.