Microbial Synthesis of Iodophile Polysaccharide by a *Clostridium* from the Cæcum of the Pig

MICROSCOPIC findings of Baker and his collaborators[1] have repeatedly confirmed the observation of Henneberg[2] that the microbial decomposition of carbohydrates in the gastro-intestinal tract may be accompanied by extensive synthesis of iodophile material within the bacterial cell. Where, as in the rumen microflora of the ox[3], biochemical observations have been attempted, they have shown that the iodophile material is a polysaccharide. A knowledge of the mechanism of such synthesis is essential, therefore, to a fuller understanding of the chemical changes accompanying the microbial breakdown of dietary carbohydrates, including starch and cellulose, in the rumen and cæcum of various animal species.

Although the taxonomic identity and growth requirements of many of the iodophile micro-organisms concerned are still unknown, in some instances they can be grown and identified in pure culture. Baker and Nasr[4], and Baker, Nasr and Morrice[5] have shown that an iodophile strain of *Clostridium butyricum* is primarily responsible for the breakdown of raw potato starch in the cæcum of the pig. The organism was isolated in pure culture on modified Beijerinck's medium[6]. Washed suspensions were then prepared according to the technique employed by Hehre and Hamilton[7] and Hehre[8] in the investigation of polysaccharide synthesis in *Neisseria pharyngitidis*, and buffered with sodium citrate. Preparations were made from young cultures, which showed little or no iodine reaction, as well as from older cultures in which the micro-organisms were replete with iodine-reacting material.

To suspensions of young cultures, glucose, glucose-1-phosphate and sucrose, respectively, were added in 2 per cent concentrations. Fructose and raffinose were also included in experiments made on older cultures. The suspensions with the various sugars added, together with control suspensions made up with buffer alone, were incubated at 37° C. A distinct, though faint, macroscopic iodine reaction was visible in the centrifuged deposit from the glucose-1-phosphate suspensions of young cells after 20 hr. After three days a strong reaction was obtained. The macroscopic findings were confirmed by microscopic examination of the micro-organisms, the contents of which were deeply stained. No reaction, macroscopic or microscopic, was observed in the control, glucose or sucrose suspensions. These observations indicated that a phosphorylating mechanism was involved in the endocellular synthesis of polysaccharide by young cells. In the older cultures the initial iodine reaction was retained in the control and glucose-1-phosphate, but disappeared in the glucose, sucrose, fructose and raffinose suspensions. The reason for the difference in behaviour between glucose-1-phosphate and the other sugars requires further investigation.

In conclusion, it may be recalled that Hehre[8] demonstrated that washed suspensions of *Neisseria pharyngitidis* synthesized iodophile polysaccharide directly from sucrose. The mechanism of synthesis, therefore, requires individual investigation for each of the isolable members of a mixed iodophile population. Thus the complex microbial facies of the gastro-intestinal tract of men and animals affords a rich province, as yet only incompletely explored, for the investigation of polysaccharide synthesis in a variety of bacteria, yeasts and protozoa.

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Participation of 'Brown Fat' Tissue in the Alarm Reaction

IN the course of our studies on the general-adaptation syndrome, we were surprised to note the intense morphological changes which occur, during the alarm reaction stage, in the so-called 'brown fat'. It will be recalled that in various animal species, a special type of adipose tissue occurs, which, upon naked-eye inspection, is characterized by its brownish colour. Histologically, it resembles a lipid-storing endocrine gland (such as the adrenal cortex, the corpus luteum or the Leydig cells of the testis), because its epithelioid cells contain numerous small lipid granules and are richly supplied with capillaries.

Such 'brown fat' (synonyms: primitive fat organ, fat gland, hibernating gland, interscapular gland, lipoid gland, cholesterol gland, glandula insularis cervicalis, multilocular adipose tissue) is normally present throughout life in certain species, such as most rodents and hibernating animals. In other species, including man, it occurs quite regularly in certain locations (for example, the vicinity of the parathyroids, thymus and kidney) during embryonic life, but later tends to become completely transformed into common adipose tissue.

In hibernating animals, the chief function of the organ appears to be the storage of certain lipids during the active season, in order to make them available throughout the hibernating period. The purpose of 'brown fat' in non-hibernating animals is rather problematical. Cramer[1] postulated a close relationship between the lipid content of this tissue and that of the adrenal cortex, emphasizing that both these organs are extremely rich in cholesterol. Sweet and Hoskins[2] found that the 'brown fat' of the woodchuck contains as much testoid material (the equivalent of 100 γ of androsterone per 50 gm. of tissue) as bull testis, the richest natural source of testoids. Vignes[3] refuses to believe that the gland acts merely as a reserve of nutritive material, since in rats even mere extirpation of the interscapular accumulation of 'brown fat' causes emaciation and death.

An intense and rapid discharge of adrenal lipids is one of the salient manifestations of the alarm reaction[4], while fat from common adipose cells is much more slowly lost under the influence of non-specific systemic stress. We now find that the discharge of adrenal cortical lipids parallels the loss of sudanophilic material from the 'brown fat'.

In a preliminary experiment, twelve adult female piebald rats (average body-weight 120 gm.) were