SOURCE OF THE ACETYL GROUP IN ACETYLCHOLINE

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It is an ill wind that blows no one any good. The advent of the ill-fated Nazi regime in the early thirties wafted many scientists from Germany into America and the United Kingdom. Among those who found hospitality in my laboratory in a mental hospital in Cardiff, Wales, was one whose major qualification for biochemical prowess was his unexpected skill in estimating acetylcholine (ACh) by the leech assay technique. He came to me about 1934 and I decided there and then to carry out studies on ACh formation in isolated brain. At that time there were very few laboratories in the world carrying out systematic biochemical studies on the brain related to brain function. Perhaps the nearest work on these lines, outside that of our laboratory, was that of Peters and his colleagues (27) in Oxford on the action of vitamin B_1 on pyruvate oxidation in polyneuritic pigeon brain preparations. Some time earlier than this we had found, in systematic studies of cerebral oxidation in brain (30) that glucose, lactate and pyruvate are readily oxidized by brain tissue in vitro and that, at normal physiological concentrations, glucose is the major fuel of the brain in vitro. It was known at that time, that brain contains an ACh-like substance and in fact, values of the order of 2 nmol/g wet wt had been quoted for the ACh content of the brains of dog, cat and guinea pig. It was not known for certain that the substance in question was ACh until it was isolated as the chloraurate from minced brain by Stedman and Stedman (31).

In 1936 we showed (29) that when rat or guinea pig brain cortex slices were incubated aerobically in a glucose-phosphate saline medium containing eserine, ACh was formed in concentrations far in excess of the amounts present in the tissue before incubation (29). The presence of eserine and

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of oxygen was necessary for the demonstration of ACh synthesis. Glucose was needed for the synthesis but the presence of either lactate or pyruvate was nearly as effective. Succinate, which is vigorously oxidized by brain, was quite ineffective in promoting ACh synthesis in incubated brain slices. The conclusion that glucose, or a breakdown product of glucose, is important for the synthesis of ACh under physiological conditions, received confirmation from MacIntosh's (16) perfusion experiments carried out on the superior cervical ganglion of the cat.

Our investigation took on a new aspect when we found that there exists in brain a substance, not pharmacologically active as far as the eserinized leech preparation is concerned, that breaks down under a variety of conditions to form ACh (17). This substance, which we concluded at the time to be the immediate precursor of free ACh, has become known as bound ACh, but we often referred to it as "combined" ACh as well as "bound" ACh. Estimates were then made of the effects of various substrates and conditions on the total ACh, i.e. on the sum of bound and free ACh, found in the tissue incubation medium at the termination of an experiment. We came to the conclusion that glucose, lactate and pyruvate were almost equally effective in causing ACh synthesis in isolated brain, that acetoacetate had a small, much lesser, effect and that succinate, acetate, succinate and α-ketoglutarate were inert as precursors of ACh (Table 1). On comparing the effects of different sugars on ACh formation in brain, incubated in the presence of KCl (31 mM) which we had observed greatly stimulates ACh synthesis in a glucose-containing medium, we found that glucose and mannose were almost equally effective in promoting ACh synthesis and that their effects were far greater than those due to equivalent concentrations of fructose or galactose (Table 2). We came to the conclusion in 1938 (17) that choline (Ch) and a metabolic product derived from the combustion in the brain of glucose, lactate or pyruvate formed "bound" or "combined" ACh which then gave rise to free ACh (Table 3).

This view was modified a little later when, from a consideration of the releasing effects of relatively high concentrations of K^+ on ACh synthesis, we concluded that an equilibrium exists in the brain cell between free ACh and combined ACh (18). We know now, of course, that acetyl-CoA (AcCoA) is required for ACh biosynthesis and that it must be the substance produced during the combustion of glucose, lactate or pyruvate in the brain that gives rise to ACh.

But almost 30 years had to elapse before the use of labelled substrates made it possible to throw more light on the origin of the acetyl group in ACh. The various substances that became seriously considered as precursors of the acetyl group were pyruvate, acetoacetate, acetate and