produced by DAB in each dietary group parallel the microsomal phospholipid changes, it may be postulated that a specific substitution of oleic for stearic acid in the lecithins of the microsomal membrane alters the arrangement of the RNA it supports such as to produce increases in the synthesis of specific proteins.

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Acetylcholine in the Electric Organ of *Torpedo*

Feldberg and Fessard[1] have shown that the electric organ of *Torpedo marmorata* is activated by acetylcholine (ACh) and that it contains a substance like ACh, as determined by bioassay, equivalent to between 40 and 100 μg ACh/g tissue. It has been suggested recently[2,3] that not all the material extracted from nervous tissue and bioassayed as ACh is in fact ACh. Since, so far as we are aware, the ACh-like activity extracted from *Torpedo* is the highest reported for any vertebrate tissue, confirmation of the nature of the active substance and a study of its metabolism in the electric organ are of interest. As a necessary preliminary we have reinvestigated the content of ACh by three different extraction procedures.

In our first experiments the tissue was extracted with acid-ethanol according to Crossland[4]. In subsequent experiments this method was compared with the usual trichloroacetic acid (10 per cent, w/v) procedure and with a perchloric acid extraction technique developed in this laboratory[5]. Samples of tissue (1 g or less) were minced with scissors in two volumes of 0·2 N perchloric acid and then rapidly and thoroughly homogenized in 'Teflon'-glass hand-operated homogenizers. The suspensions were centrifuged, the supernatants stored at 4° C and the precipitates re-homogenized three times with two volumes of water and recentrifuged. The supernatants were combined and brought up to *p*H 4 with 0·2 N sodium hydroxide. The extracts, in a final dilution of 1 in 100, were assayed against acetylcholine chloride on the frog rectus abdominis muscle, guinea-pig ileum and the dorsal muscle of the leech; the activity was the same on all three test objects. Alkali-boiled controls were used in all assays The average values from two series of experiments in which perchloric acid and acid-ethanol were used, and where perchloric acid and trichloroacetic acid were compared, are shown in Table 1. These results were from extractions performed at 0°–10° C. The yields were lower when the tissue was initially frozen with liquid nitrogen, an observation in agreement with Hobbiger and Werner[6]. The very low activities obtained with acid-ethanol can be attributed to the failure of the extractant to inactivate the tissue cholinesterase completely. If the minced tissue was left in contact with eserine (10^{-4} M) or diisopropylphosphorofluoridate (10^{-4} M) at 0° C for 30 min or longer before extraction with acid-ethanol the ACh-levels were comparable with those obtained using perchloric acid. The range of activities obtained with perchloric acid was between 65 and 130 μg ACh/g tissue, suggesting that the values reported by Feldberg and Fessard[1] may be on average too low rather than too high. Since one of the objects of the investigation was to characterize chemically the active substance which by bioassay appears to be ACh, perchloric acid extraction seems to be the method of choice. Trichloroacetic acid has the disadvantage that the procedure is more time consuming. Moreover, it does not precipitate phospholipids so effectively[7] and may interfere with the chromatography of ACh[8]. Results of the chemical investigation will be published separately.

Table 1. Comparison of Methods of Extraction of Acetylcholine from the Electric Organ of *Torpedo*

	ACh content (μg/g tissue)		
	perchloric acid	acid-ethanol	trichloroacetic acid
Series 1	86	25	—
Series 2	70	—	68

We have some evidence that endogenous synthesis of ACh may occur both in the excised whole organ and in minced tissue. Samples of tissue allowed to remain at room temperature for periods of up to 2 h contained more ACh activity than those extracted immediately the fish was killed. The largest increase was equivalent to 60 μg ACh/g tissue in a sample left for 1 h at room temperature. No increase in ACh activity was found in samples stored at 0° C. *In vitro* estimation of choline acetyltransferase activity in cysteine-sucrose tissue homogenates showed that the enzyme was capable of a rate of ACh synthesis of 5–8 mg ACh/g tissue/h.

These experiments largely confirm the original observations of Feldberg and Fessard[1]. In addition a new and convenient method of extraction has been developed which forms a basis for further study of the metabolism of ACh in the electric organ of *Torpedo*.

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Red Pigments of *Dactynotus rudbeckiae* and *D. ambrosiae* [Homoptera, Aphididae]

The extensive investigations of Lord Todd and his colleagues[1] have shown that several related perylenequinone pigments, the erythroaphins (Fig. 1), can be obtained from many aphid species; the two most completely investigated representatives, the erythroaphins *fb* and *sl*, are stereoisomers of the formula $C_{30}H_{22}O_8$. These pigments, however, are not present in the live insect, but are formed through post-mortem enzymatic transformations of yellow precursors, the protoaphins, which do not contain the perylene nucleus[1a]. In the course of research on naturally