advantageous in making accurate measurements. Since the amplitude of the audiofrequency signal is changed by the deflecting force, the base line reflects changes in the signal source and the position of the rotor. Direct-current amplification is not required to obtain static load characteristics and calibration can be easily obtained by the use of laboratory weights hung on the lever arm.

The frequency of the oscillator used to power the selsyn can be used as a time signal when it is set so that the individual peaks are visibly separated by the sweep speed (Fig. 2b). This is particularly advantageous when using cathode-ray technique, since it eliminates the requirement of additional timing systems. The natural frequency response of the device described was determined to be 600 cps with excellent damping (Fig. 2c). Higher frequency response can be obtained by sacrificing sensitivity by the use of a stiffer spring and vice versa. The unit described has a shaft 0.125″ in diameter and is not adapted to more than a few kilograms of load. Larger units, such as the type GE 2J1G1, have been tested and are capable of withstanding higher loads. The size 5 selsyn, also readily available from Army surplus equipment dealers, has a 5/16″ shaft and is adapted to loads encountered in myographic observations on man.

Effect of Calcium Chloride on the Preparation of Extracts of *H. pertussis*[1]

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It is well known that mucin-like substances interfere with the separation and purification of tissue proteins (*1*). Difficulty is also encountered in the isolation of toxins and other antigens from mucin-producing bacteria, since the mucin-like materials hinder effective centrifugation, filtration, and washing.

During the course of studies on the isolation and characterization of toxic and somatic antigens from a toxin-producing strain of *H. pertussis* (*7*), contaminating mucin-like substances greatly hindered all preparative procedures. Sharp separation of the bacteria from their soluble antigens by either centrifugation or filtration was almost impossible, and extracts prepared by either of these methods were found to be unsatisfactory for further purification of the toxin. Under certain conditions, the toxin was strongly, and at times irreversibly, adsorbed by the mucin. It was thus impossible to determine the true solubility of the toxin.

It was observed that the addition of optimal amounts of $CaCl_2$ to a suspension of *H. pertussis* results either in the precipitation or in a physiochemical alteration of the mucin-like substances with no loss or destruction of pertussal toxin. The results of a typical experiment are given in Table 1. It will be seen that the presence of

TABLE 1

Extracting fluid	Appearance of supernatant	Mg of N/ml	MLD/ml
Distilled water	Turbid and viscous	0.72	160
0.05 M $CaCl_2$	Translucent; only slightly opalescent; straw colored	0.49	160
0.1 M $CaCl_2$	Translucent; slightly opalescent; straw colored	0.54	160

0.05 M–0.1 M $CaCl_2$ in a 2.5% suspension of *H. pertussis* gives a translucent, slightly opalescent, straw-colored solution which contains all of the toxin originally present in the suspension. Furthermore, it will be noted that the nitrogen content of these $CaCl_2$ extracts is considerably lower than that of the aqueous extract, indicating that the elimination of nitrogenous impurities is also achieved at this step.

Removal or alteration of the mucin greatly influences the solubility of the toxin in methanol-water mixtures under controlled conditions of pH, ionic strength, and temperature (*2–6*). In aqueous extracts, the toxin is insoluble between pH 4.0 and 4.8 at low ionic strength and is highly soluble at hydrogen ion concentrations below pH 5. All precipitates are mucilaginous and dissolve with great difficulty in either water or salt solutions. In $CaCl_2$ extracts, on the other hand, the toxin is soluble between pH 4 and 5, but is quantitatively precipitated between pH 5 and 6.4 under appropriate conditions of methanol concentration, temperature, and ionic strength. These precipitates are flocculent and dissolve readily in salt solutions.

While every protein or bacterial suspension will present a new problem, it is possible that the controlled use of multivalent cations or anions will be of aid in the removal of mucin-like contaminants from other proteins which require purification and characterization. Work is in progress in this laboratory on the mechanism of the effect of $CaCl_2$ and on the influence of inorganic salts containing multivalent cations and anions on other protein systems.

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