essentially the same manner as previously published[4,5] and contained approximately 20 Riddle-Bates units per mg. L 269 also caused lactation in normal, virgin, post-oestrus guinea pigs in a total dose of 4 mg or less. The hormone was dissolved with the help of a small amount of acid or alkali and dialyzed against the desired buffers for 24 hours or more until there was no difference in the pH and conductance between the dialysate and the buffer. The ionic strength of the phosphate or acetate buffer solutions was 0.055 in all experiments. The potential gradients were kept practically constant (*ca.* 9 volts/cm).

The schlieren photographs, taken at 15-minute intervals in a typical experiment with a 0.5 per cent. solution, are shown in Fig. 1. They show only one sharp boundary throughout the experiment. Since Tiselius[6] has demonstrated that 0.02 per cent. protein solution can be detected by electrophoresis, the single boundary shown by L 269 indicates that in all probability no great amount of contaminant, if any, is present.

The results of mobility studies made with 0.2–0.3 per cent. solutions of L 269 are recorded in Fig. 2. The + and − refer to charge on the protein, which is presumably the hormone. It will be seen that the iso-electric point of the preparation falls at pH 5.70. It is interesting to note that Shipley *et al.*[2] have reported an iso-electric point of approximately pH 5.6 for their preparation, although of the 4 electrophoresis experiments made with their crystalline prolactin, only 2 were made with "native" substance.

While it may be concluded that the single sharp boundary observed in these electrophoretic studies of our lactogenic preparation is suggestive of its purity, a more decisive conclusion may be reached from solubility studies now in progress.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DETERMINATION OF CAROTENE

IN the study of plant pigments two methods were developed for the quantitative determination of carotene. Willstätter[1] used a two-phase solvent extraction method for separating carotene from xanthophyll and chlorophyll. Tswett[2] made use of an adsorbing column through which he passed a solution of plant pigments forming a chromatograph of the plant pigments with complete separation of carotene.

Many modifications of these two methods have been developed since Moore[3] discovered that carotene was converted into vitamin A. Schertz[4] modified Willstätter's method so that carotene, xanthophyll and chlorophyll could be determined quantitatively. Guilbert[5] modified Schertz method so that carotene determinations could be made more rapidly. Peterson, Hughes and Freeman[6] modified Guilbert's method by eliminating several unnecessary steps which further reduced the time required for making carotene determinations. Strain[7] used a modification of Tswett's method for obtaining carotene in crystalline form.

In the routine analysis of a large number of samples of dehydrated alfalfa for the quantitative determination of carotene any modification of the Willstätter method required considerable mechanical manipulation. Tswett's method, although requiring a longer time for the extraction of the plant pigments, is quite simple, rapid and accurate. For the past several years we have been using a modification of Tswett's method for the determination of carotene in dehydrated alfalfa.

Briefly, this method consists of placing a one-gram sample of alfalfa in a flask, to which is added 100 cc of petroleum ether. The flask is stoppered and set aside over night. The petroleum ether solution of plant pigments is poured on a column of finely divided soda ash and drawn through the column by the aid of suction. A chromatograph of the various plant pigments forms on the column with the separation of carotene, which passes through with the petroleum ether. Some of the petroleum ether is absorbed by the soda ash so that it is necessary to add fresh petroleum ether until it comes through clear in order to elute all the carotene from the column. The filtering column is made of a filter tube with a small plug of cotton in the bottom upon which is packed soda ash. Tswett recommended the use of MgO or $CaCO_3$. We find, however, that soda ash is to be preferred to any other adsorbent which we have used.

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