

then gives diglucoylamine (cf. hydrolysis experiments). The failure of ammonium salts of the stronger mineral acids to exhibit these phenomena may well be due to the non-availability of the free ammonia required for the first step.

The interpretation of paper chromatograms and of optical rotation measurements is rendered complex by the instability of glucoylamine, and by mutarotation effects, respectively; but we believe that valuable data are still to be gained from each method. A more detailed account of the experiments mentioned above and of current investigations will be given later. Meanwhile we wish to emphasize that chromatograms of sugars in biological fluids, and in other solutions which may contain nitrogenous matter, should be interpreted with considerable caution.

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Estimation of Corticosteroids

THE use of tetrazolium compounds for the detection of certain-reducing steroids on paper chromatograms¹ prompted an inquiry into the possible application of these compounds to quantitative work, and a method of estimating corticosteroids has now been developed using 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl-tetrazolium chloride. Earlier attempts² to utilize the intense blue colour formed on reduction of this substance in alkaline solution were not entirely successful, since it was not found possible to stabilize the colour sufficiently for routine work. The method has therefore been modified so as to make use of the more stable but slightly less intense red colour produced on neutralization of the blue reaction mixture. In addition, pyridine has been replaced by *isopropanol* as a more convenient solvent.

The modified procedure is as follows. Samples containing 5–50 $\mu\text{gm.}$ of corticosteroid are pipetted into test-tubes and evaporated to dryness *in vacuo*. 0.2 ml. of a 1 per cent solution of the reagent in ethanol (freshly prepared) is added and mixed well to ensure solution of the steroid. A 'blank' tube is also set up. The tubes are placed in an incubator at 25° C. for 10–15 min., after which 0.5 ml. of *N/20* sodium hydroxide in 25 per cent *isopropanol* (also warmed to 25° C.) is added. After thorough mixing the tubes are returned to the incubator for 15 min. The reaction is then stopped by addition of 3 ml. of *N/100* acetic acid in 90 per cent *isopropanol* and the extinctions are measured at 480 $\text{m}\mu$. The relation between extinction and quantity of steroid is linear

over the range 5–50 $\mu\text{gm.}$ for cortisone, 17-hydroxy-corticosterone, deoxycorticosterone and 17-hydroxy-deoxycorticosterone, the extinctions (1-cm. cells) for 10 $\mu\text{gm.}$ of each steroid being 0.13, 0.14, 0.11 and 0.14 respectively. The method appears to be slightly more sensitive than that of Chen and Tewell³, who used 'blue tetrazolium BT', which was not available when the present work was begun. It has been used satisfactorily for the estimation of corticosteroids in fractional chromatographic work.

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Determination of the Configuration of Silk Fibroin Dissolved in Aqueous Solutions of Lithium Bromide

FOLLOWING the discovery, made earlier in this laboratory¹, that water-soluble silk fibroin is predominantly α -folded, it was natural to speculate upon the molecular configuration of this protein in the aqueous solutions of lithium bromide from which it can be isolated in the soluble form and in which *inter-chain* hydrogen bonds clearly do not persist. Recently, the difficulties of making the necessary measurements of the absorption of infra-red radiation were lessened significantly by the preparation of remarkably clear, stable solutions of high protein content (25–50 gm. of fibroin per 100 ml.). In agreement with observations reported by von Weimarn² and others³, it was found that these 'silk syrups' could readily be made at ordinary temperatures either by dispersing fibroin in suitable solutions of lithium bromide or by a process of 'salting-out' (which was indistinguishable, qualitatively, from the separation of phases observable in many simpler polymer-penetrant systems).

Two typical syrups, *A* and *B*, have been prepared; they have the following compositions (by weight):

Fibroin	<i>A</i>	<i>B</i>
Lithium bromide	15	16
Water	46	50
	39	34
	100	100

The fibroin used in this work was obtained by thoroughly 'degumming' a specimen of *Bombyx mori* silk of Syrian origin. Syrup *A* was made by introducing 3.0 gm. of fibroin into a cooled solution of 9.2 gm. of lithium bromide in 7.8 gm. of water in a test-tube. The tube was then tightly corked and partially immersed in water kept at 25.0° C. ($\pm 0.1^\circ$). Equilibrium was established within 24 hr.: the product was a homogeneous viscous liquid. Syrup *B*, the composition of which had to be found by microchemical analysis, was obtained as the upper of the two liquid layers which separated as equilibrium was established at 25° C. in a system having the following