and the daily outgrowth of each culture was recorded for a period of 10 days. At the close of the experiment, 109 cultures planted with broth; 105 planted with extract from frozen embryos; and 103 controls utilizing fresh embryo juice, had lived for 10 days or longer. These cultures form the basis for the results recorded in Table I and Graph 2.

Microscopical study of each of the cultures in the experimental series showed no marked differences or variations in the behavior of the cells or any structural changes which would differentiate these cells from those in the control series. Many dividing cells were observed and an abundant outgrowth took place in all the cultures. Graphs 1 and 2 are self-explanatory. Examination of Table I indicates that increased growth of tissue cultured in 1% chick embryo-broth, is not sufficiently greater than that of tissue grown in fresh embryo juice to make the former medium of any particular value except as a matter of convenience during periods when fresh embryos are scarce. On the other hand the difference in growth of cultures utilizing embryo juice made from frozen embryos compared with the growth of cultures in juice from fresh embryos, is so much greater as to be of real significance. Furthermore, the use of juice made from frozen embryos precludes the possible contamination of cultures due to the introduction of viable cells from insufficiently centrifuged fresh extracts.

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The Biuret and Ninhydrin Tests for Proteins as Measured with Hardy's Spectrophotometer.

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Color tests for proteins have long been in use, although in most cases the chemistry involved is not clearly understood. One method of attack upon this problem is to analyse the transmission spectra of the colored solutions with the aid of Hardy's recording photoelectric spectrophotometer. This has been done for the ninhydrin and biuret reactions.

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The ninhydrin test was performed according to Koch[1] by adding 2.5 cc. 0.1% ninhydrin to 10 cc. protein solution, and boiling for one minute. The cloudy blue to red solutions were filtered before measuring their transmission of light in the various regions of the spectrum. For the ninhydrin test on 10% bacto-peptone (Fig. 1, upper curve) the percent transmission of light by the colored solution increases continuously from 2% at a wavelength of 400 mμ to 88% at 700 mμ. A very similar curve is obtained for the ninhydrin test

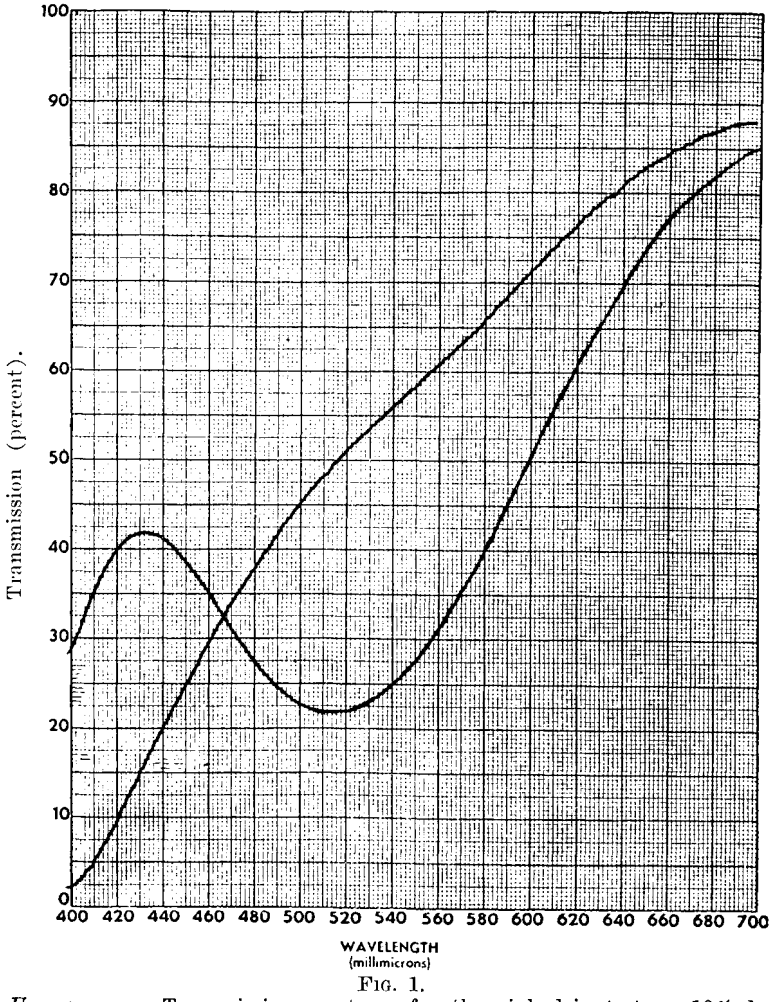

FIG. 1.

Upper curve: Transmission spectrum for the ninhydrin test on 10% bacto-peptone.

Lower curve: Transmission spectrum for the biuret test on 20% casein.

[1] Koch, F. C., *Practical Methods in Biochemistry*, Baltimore, 1937.

on 20% casein (Hammarsten). Digestion of these 2 proteins by 0.25% trypsin (Fairchild and Foster) at about pH 8 and at 50°C. caused very little change in the shape of the curve, with a slight decrease in percent transmission over the middle of the spectrum. In all cases where digestion was studied, the course of the reaction was followed by means of the formal titration. Curves for the increase in carboxyl groups as a function of time were very similar to those obtained by Northrop and Kunitz.[2]

The biuret reaction was performed according to Koch[1] by adding 5 cc. 10% NaOH to 5 cc. protein solution, and then adding 20 drops 1% $CuSO_4$. The cloudy or clear red to purple solutions were filtered before analyzing with the spectrophotometer. The test was performed on 2 preparations of 20% casein, a 10% solution of bactopeptone, 3.5 and 5% egg albumen, and 5% edestin. Similarly shaped colorimeter curves were obtained for all these proteins with a maximum at 431 mμ, a minimum at 514 mμ, and a second maximum at about 700 mμ (Fig. 1, lower curve). The actual percent transmission at a given wave length, and also the shape of the curve, varies with the nature and concentration of the respective proteins.

The biuret reaction was also performed according to Lieben and Jesserer[3] by adding 6 cc. 10% NaOH plus 4 cc. 10% $CuSO_4$ to 10 cc. 1% protein. The heavy precipitate was removed by filtering. Only the 2 egg albumen and the edestin solutions were used. The shape of the colorimeter curves was very similar to that obtained for the Koch biuret. The transmission curves are somewhat displaced toward the region of higher wave lengths so that the first maximum now comes at 437 mμ, the minimum at 552 mμ, while the second maximum is off the scale. The intensity and shade of color in the biuret reaction are similar but not identical for different proteins at equal concentrations, although Lieben and Jesserer[3] believe they are the same.

The spectrophotometer reveals the fact that the violet biuret color is made up of 2 components, red and blue. By decreasing the amount of 1% $CuSO_4$ added to 2.5% egg albumin in 5% NaOH from 40 to 10 drops the blue component of the percent transmission curve practically disappears. The red component, however, is almost unaffected by this procedure. It is suggested that the red color is due to the presence of a copper-protein complex, while the blue color may be caused by the copper ions left free in the solution. The similarity between the blue portion of the curve and the percent

2 Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1932, **16**, 295.

3 Lieben, F., and Jesserer, H., *Biochem. Z.*, 1936, **285**, 36.

transmission curve obtained with a saturated $CuSO_4$ solution supports this suggestion. From a study of extinction coefficients Lieben and Jesserer[3] also concluded that the biuret color consisted of red and blue components. They also report that if the biuret mixture is carefully acidified, the red component disappears before the blue.

The biuret test is specific for peptide linkages in proteins, and is also given by such compounds as biuret and malonamide (Schiff[4]). A typical protein biuret curve was obtained with maxima at 420 and 700 mμ, and a minimum at 520 mμ, for a solution containing 1.25% biuret, 5% NaOH, and 30 drops 1% $CuSO_4$. A similar solution containing 2.5% malonamide gave a blue maximum at 445 mμ, but the red portion of the curve was almost completely missing. Biuret apparently represents the type of grouping in proteins responsible for the biuret test better than does malonamide.

The digestion of 20% casein, 10% bacto-peptone, 3.5 and 5% egg albumen, and 5% edestin by 0.25% trypsin was studied at 50°C. and pH about 8. The formal titration curves were similar to those obtained in the ninhydrin experiments. Biuret tests were performed on the digest at successive intervals and analyzed with the spectrophotometer. A comparison of the transmission curves of the biuret tests indicated that during the course of digestion there is relatively no change in the positions of the 2 maxima and the minimum. There was considerable fluctuation in the percent transmission of the biuret solution at a given wave length, however. These fluctuations during digestion were more marked in the blue portion of the curve than in the red. There was no consistent increase or decrease in percent transmission during hydrolysis, when the biuret test was performed by the 2 different methods. The cause of the sharp rise or fall in percent transmission in various parts of the spectrum during digestion is not known. Changes in the colloidal properties of the protein solution might possibly be responsible.

Different proteins give essentially the same ninhydrin and biuret tests as measured with the spectrophotometer, and the general shape of the transmission curves is not changed by tryptic digestion of the proteins.

[4] Schiff, H., *Berichte,* 1896, **29**, 298, 354.