with x-rays on tissue cultures of chick fibroblasts. Vit. K substitute alone and in combination with x-rays, it was observed, produced mitotic inhibition. In general in our studies it was observed that the treated roots besides showing an inhibition in linear growth had a lower mitotic percentage than the controls though it must be emphasized that no karyokinetic abnormalities were observed.

Summary. The effect of folic acid, aminopterin and vitamin K substitute dissolved or suspended at given concentrations in water, on the growth and development of roots from bulbs of *Allium cepa* was investigated. Folic acid at concentrations of 10^{-5}, 10^{-7} and 10^{-9} g/ml, aminopterin at concentrations of 10^{-7}, 10^{-9} and 10^{-10} g/ml and vitamin K substitute at concentrations of 10^{-4}, 10^{-5} and 10^{-7} g/ml inhibited the linear growth of roots of *Allium cepa*. The percent of mitosis in the treated roots was in general lower than that of the controls. No abnormalities of the mitotic figures in the treated root tips were observed. The inhibition of growth by these agents may be due partially to an interference with the process of cellular elongation and differentiation following karyokinesis and partially by reducing the rate of nuclear division.

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Biuret Reaction as Applied to Determination of Antibody Nitrogen. (17888)

M. SOLOTOROVSKY, C. C. PORTER, AND R. H. SILBER
(Introduced by H. Molitor)
From the Merck Institute for Therapeutic Research, Rahway, N. J.

The quantitative biuret reaction(1) has been successfully applied to the determination of protein in urine(2), blood serum(3) and cerebrospinal fluid(4). Since the method is simple and rapid, it appeared desirable to investigate its use in immunochemical procedures. Various proteins may yield biuret colors whose absorption maxima differ slightly in intensity and position(5). In a series of determinations of optical density per mg N at 540 mμ relative to bovine serum albumin, we obtain values of 0.95, 0.98, 1.08, and 1.12, respectively for horse, rabbit, rat and dog serum, 1.01 for crystalline egg albumin, and 0.98 for bovine γ-globulin. With antigen-antibody precipitates in which the components give similar absorption maxima, the error encountered should be negligible. Correlation with nitrogen content may be obtained by the use of proper standardization procedures.

Materials and methods. Antigens. Crystalline egg albumin and crystalline bovine albumin were used in these studies. For immunization procedures, the albumins were precipitated with alum; for antibody precipitation, they were dissolved in 0.85% NaCl solution. The nitrogen contents were determined by the micro-Kjeldahl method. *Antisera**. Albino rabbits weighing about 1.5 kg were given 4 series of 4 intravenous injections of antigen increasing from 1.5 mg to 7.5 mg. Rest periods of 3 days were allowed between series, and 7 days after the last injection blood was withdrawn aseptically. Sera were preserved by the addition of 0.01% sodium merthiolate. *Precipitation of antibody.* Antibody was precipitated according to the method of Heidelberger and Kendall(6) as described by Kabat and Mayer(7). *Biuret reaction.* The biuret reagent described by

1. Autenrieth, W., and Mink, F., *Münch. med. Wochschr.*, 1915, v62, 1417.
2. Hiller, A., PROC. SOC. EXP. BIOL. AND MED., 1928, v24, 385.
3. Robinson, H. W., and Hogden, C. G., *J. Biol. Chem.*, 1940, v135, 707.
4. Dittebrandt, M., *Am. J. Clin. Path.*, 1948, v18, 439.
5. Sizer, I. W., PROC. SOC. EXP. BIOL. AND MED., 1937, v37, 107.

* The antisera were prepared by Miss E. Jane Tullius.

Kingsley(8), was prepared by mixing 400 ml of a 1% aqueous solution of copper sulfate pentahydrate with 2000 ml of a 1.4% aqueous solution of sodium hydroxide. Analytical reagent grade chemicals were used. The reagent is stable for several months when protected from light.

To the antigen-antibody precipitates were added 1 ml of water followed by 6 ml of biuret reagent. The solutions were then transferred to ½ inch square cuvettes and their optical densities (–log T) were compared with that of a water-reagent blank at 540 mu. A Coleman Universal Spectrophotometer, Model 11 was used. A dried sample of crystalline bovine albumin† (fraction V, Armour) was employed as a reference standard. With the biuret reagent, this protein yielded solutions with optical densities of 0.302 per mg N. Precipitates from rabbit anti-bovine albumin serum plus homologous antigen, containing 0.2 to 0.7 mg N (micro-Kjeldahl), yielded average optical density values of 0.309 per mg N. In a series of measurements with similar precipitates, the optical density per mg N for the bovine albumin was multiplied by the ratio 0.309/0.302 to obtain optical density per mg N for the precipitates.

Kjeldahl method. A micro procedure with selenium(9) as the digestion catalyst was used. This catalyst may yield unreliable results(10), but in the present work nitrogen values obtained with the selenium catalyst were similar to those obtained with a copper catalyst.

Results. Data on the antibody N content of 7 rabbit antisera, as determined by the biuret and Kjeldahl methods, are given in Table I: one was an anti-bovine albumin serum and the 6 others were anti-egg albumin sera. With 3 of the 7 sera the 2 methods yielded values differing by 0.02 mg antibody N per ml of serum, while differences of 0.01 mg or less were obtained with the 4 remaining sera. Thus analysis of duplicate precipitates by the two methods agreed within ± 0.02 mg for samples containing 0.1 to 0.5 mg of antibody N.

TABLE I.
Comparison of Antibody Nitrogen Determinations with the Biuret and Kjeldahl Methods.

Rabbit antiserum No.	Antibody N, mg/ml serum: Biuret	Kjeldahl
Anti-bovine albumin 1	.46	.46
	.48	.47
" " " 1	.45	.45
(10% less antigen in antigen-antibody mixture)	.45	.45
Anti-egg albumin 1	.50	.52
	.51	.51
	.51	.52
" " " 2	.27	.27
	.26	.26
" " " 3	.31	.29
	*	.29
" " " 4	.23	.24
	.22	*
" " " 5	.26	.28
	.29	.26
" " " 6	.10	.12
	.11	*

* Tube broken during centrifugation.

Summary and conclusions. A quantitative biuret method for the determination of protein has been applied to the measurement of antibody N. The nitrogen contents of duplicate antigen-antibody precipitates from rabbit anti-bovine albumin serum and rabbit anti-egg albumin sera, plus the homologous antigens, were determined by the biuret and micro-Kjeldahl methods. The results agreed within ± 0.02 mg nitrogen with precipitates containing 0.1 to 0.5 mg antibody nitrogen.

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6. Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, v62, 697.

7. Kabat, E. A., and Mayer, M. M., Experimental Immuno-chemistry, C. C. Thomas, Springfield, Ill., p. 59.

8. Kingsley, G. R., *J. Lab. and Clin. Med.*, 1942, v27, 840.

† 15.2% N by the micro-Dumas method. This determination was performed by Mr. R. N. Boos, Research Laboratories of Merck and Co., Inc.

9. Lauro, M. F., *Ind. Eng. Chem. Anal. Ed.*, 1931, v12, 657.

10. Broadstreet, R. B., *Ind. Eng. Chem. Anal. Ed.*, 1940, v12, 657.