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Reaction of Folin's Reagent with Proteins and Biuret Compounds in Presence of Cupric Ion.

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In an earlier paper[1] the writer noted that traces of copper sulfate increased the blue color obtained by treating such proteins as pepsin or gelatin with Folin's phenol reagent.

It has now been found that a number of organic substances reduce the Folin's phenol reagent only if a small amount of cupric ion is present. These same substances also show a positive biuret reaction.

The copper-phenol values in Table I represent the amount of color per milligram of substance produced as a result of adding M/1300 copper sulfate to the solution under examination with the phenol reagent. One obtains this value from the difference in color values produced with and without copper ion. For many substances no color is produced in the absence of copper but most proteins contain tyrosine and tryptophane and must, therefore, be analyzed with and without cupric ion. No attempt has been made to determine whether each of the substances in Table I produces a blue color exactly in proportion to its concentration. With leucyl-glycyl-glycine the proportionality is quite good while with pure biuret it is not as good. The order of magnitude of the values in Table I is of more significance than the absolute values. The solutions have been read against a tyrosine standard and the values calculated as the number of milligrams of tyrosine that will give the same amount of color under identical conditions.

No amino acids show any comparable effect of the presence of cupric ion when tested with the phenol reagent.

With certain substances, particularly biuret, the presence of some non-chromogenic substances has fairly pronounced effect on the amount of blue color that develops in the presence of cupric ion. Thus the presence of glycine or glycyl-glycine increases the copper-phenol color of biuret. Urea gives a blue color with the phenol reagent in the presence of copper ion and will, therefore, interfere if present in a solution being analyzed.

[1] Herriott, R. M., *J. Gen. Physiol.*, 1935, **19**, 287.

TABLE I.

Substance	Amount used, mg	Copper-phenol color value per mg material,* mg tyrosine
Tyrosine	0.15	0
Biuret†	0.1	1.3
Casein (Hammarsten)	0.84	.13
Zein	0.81	.17
Egg albumin	1.05	.15
Edestin	0.74	.23
Gliadin "B"	0.63	.22
Pepsin	0.53	.20
Pepsin (autolysate)	0.53	.24
Pepsin (acid hydrolysate)	1.0	.02
Gelatin	1.0	.14
Gelatin (acid hydrolysate)	10.5	.02
Peptone (Roche)	0.5	.12
Glycyl-tyrosine	0.18	.0
Glycyl-tryptophane	0.4	.0
Glycyl-leucine†	18.9	.005
Glycyl-glycine†	13.2	.015
Malonamide†	1.0	.25
Oxamide†	0.9	.20
Formamide†	4.5	.0
Leucyl-glycyl-glycine†	0.25	.2
Histidine	4.6	.02

*This value is obtained as the difference between the value in the presence of copper ion and in the absence of it, divided by the milligrams of material used.
†These substances show practically no color in the absence of copper ion.

In view of the fact that the products of hydrolysis have little if any influence on the color of leucyl-glycyl-glycine, it seems likely that one could follow quantitatively the extent of enzymatic hydrolysis of this and similar peptides by means of this color test.

The amount of copper required for maximal color development varies somewhat with different substances but it has been found that 1 ml of M/100 copper sulphate is maximal or nearly so for most of the different substances thus far used.

Very little can be said about the mechanism of the reaction but it should be recalled that Wu[2] pointed out many years ago that as little as one part in five million of cuprous copper ion reduces the phenol reagent.

These experiments demonstrate that under certain conditions proteins or peptides which seem unreactive may, in the presence of very small amounts of a second substance, exhibit very marked reactivity.

Experimental Details. Procedure. One ml of a solution of the substance to be studied was put into a 50 ml Erlenmeyer flask along

2 Wu, H., *J. Biol. Chem.*, 1920, **43**, 189.

with 1 ml of M/100 copper sulphate and 8 ml of 0.5 N sodium hydroxide. To this was then added dropwise with whirling 3 ml of the ⅓ dilution of Folin's[3] phenol reagent. The solutions were then read after 5-10 minutes against 0.15 mg tyrosine treated in a similar manner.

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Use of Anterior Lobe of Prostate Gland in the Assay of Metakentrin.

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We believe that the recent isolation of a homogeneous protein, from pituitary tissue, which has the property of causing the formation of corpora lutea from preformed graafian follicles and of stimulating ovarian and testicular interstitial cells in rats establishes the individuality of a gonadotrophin with some but not all the properties of luteinizing hormone as originally recognized by Fevold, Hisaw and their coworkers. During the course of our purification procedures, we have utilized the weight increase of the anterior lobe of the prostate gland in the assay of the interstitial cell-stimulating hormone[1, 2, 3]—metakentrin.[4] The principal objection to this procedure lay in the possibility that the substance stimulating the secretion of androgen by the interstitial cells of the male gonad was not identical with the substance causing formation of corpora lutea. Following the isolation of metakentrin[1] we have determined that under proper conditions all these biological effects are manifestations of this pure preparation. We are prompted, therefore, to record our experience and technic in assaying this hormone by the ventral prostate method.

Our standard procedure is as follows: The animals used are hypophysectomized immature male rats. The operation is per-

[3] Folin, O., and Ciocalteau, V., *J. Biol. Chem.*, 1927, **73**, 627.

[1] Shedlovsky, T., Rothen, A., Greep, R. O., van Dyke, H. B., and Chow, B. F., *Science*, 1940, **92**, 178.

[2] Chow, B. F., Greep, R. O., and van Dyke, H. B., *J. Endocrinol.*, 1939, **1**, 439.

[3] Greep, R. O., Chow, B. F., and van Dyke, H. B., *J. Biol. Chem.*, 1940, **133**, 289.

[4] Coffin, H. C., and van Dyke, H. B., *Science*, 1941, **93**, 61.