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Color Reaction for Certain Amino Acids, Amines, and Proteins

Abstract. Proteins, certain amino acids, and amines undergo a potentially useful color reaction. The reaction involves the apparent formation of pyrroles when the compounds are allowed to react with acetonylacetone. The pyrroles yield colored complexes on coupling with *p*-dimethylaminobenzaldehyde. This report describes the specificity and possible uses of this reaction in colorimetric measurements and in paper chromatographic detection of these compounds.

In experiments measuring hexosamine by the Elson-Morgan reaction (*1*) in various crude extracts, it was found that substitution of acetonylacetone for acetylacetone gave values much higher than expected on the basis of the indole HCl (*2*) or standard Elson-Morgan (*1*) methods. Preliminary results (*3*) indicated that this was not due to a greater color yield per unit of hexosamine, for, with a standard glucosamine sample, the use of acetonylacetone resulted in less color (about one-half) than use of acetylacetone. This suggested the possibility that the acetonylacetone reacted with a wide variety of amino compounds to form pyrroles (*4*) which were subsequently available for coupling with *p*-dimethylaminobenzaldehyde.

A study of the specificity (*3*) toward a number of compounds was undertaken. Amino acids, sugars, organic acids, purines, pyrimidines, amines (primary, secondary, and tertiary), and many miscellaneous compounds were tested. None of the sugars glucose, galactose, fucose, and rhamnose, at concentrations as high as 10 mg/ml, gave appreciable color. Citric acid at the same level gave no color. The following purines, pyrimidines, amino acids, amines, and miscellaneous compounds, at 0.5 to 2 mg/ml levels (or saturation for those of low solubility), gave little or no color: xanthine, allantoin, adenine, creatine, guanine, hemin, uracil, urea, ribonucleic acid, triethanolamine, choline chloride, diphenylamine, aspartic acid, leucine, phenylalanine, glutamic acid, proline, cystine, methionine, isoleucine, valine, tyrosine, and tryptophan. In Table 1 are given the relative light absorptions at 530 mμ, of the compounds reacting positively. The color from compounds giving positive results was red, with the exception of aniline, which gave a very intense yellow color. The following proteins in concentrations as low as 1 mg/ml also gave fairly intense red complexes: gelatin, egg albumin, lysozyme, and gastric mucin. It is evident that the sensitivity varied widely, being particularly high with certain amino acids, amines, and proteins.

The positive reaction found for NH_4OH (Table 1) is quite interesting and is included to indicate that precautions must be taken to exclude it; exclusion is also necessary when Ninhydrin is used (*5*).

The conclusion that the color developed with the amino acids, amines, and proteins was due to a coupling of *p*-dimethylaminobenzaldehyde with some product (perhaps pyrrole) formed by a reaction with acetonylacetone (but not with acetylacetone) is supported by the following control determinations. Direct treatment of the compounds with *p*-dimethylaminobenzaldehyde without prior acetonylacetone treatment gave no color other than a light violet with gastric mucin and a very slight yellow with tryptophan, citrulline, and urea. Treatment with acetonylacetone without heat gave similar results. Treatment with *p*-dimethylaminobenzaldehyde preceded by a heating period in the presence of Na_2CO_3 but without acetonylacetone also gave the same results. Use of the standard Elson-Morgan method with acetylacetone on the same compounds yielded only the typical red color with glucosamine and a slight yellow with tryptophan, citrulline, and urea.

The potential usefulness of this reaction stems from the following. First, the absorption spectra of representative materials—egg albumin, gastric mucin, lysozyme, lysine, arginine, glycine, tryptamine, histamine, and ethanolamine—all show peaks at 530 mμ. The light absorption at this wavelength is linear with concentration for the amino acids below 0.1 to 0.2 mg/ml, for the proteins below 1 to 2 mg/ml, and for the amines below 0.05 to 0.1 mg/ml.

Table 1. Relative color intensities of compounds reacting positively in the acetonylacetone test.

Compound	Optical density/mg at 530 mμ
Alanine	0.374
Histidine	0.406
Arginine	0.720
Lysine	1.10
Threonine	0.865
Glycine	2.00
Serine	0.285
Ornithine	1.70
Tyramine	1.60
Citrulline	0.595
NH_4OH	0.950
Ethanolamine	1.70
Ethylenediamine	1.90
Ethylamine	1.90
Tryptamine	1.70
Histamine	1.70
Glucosamine	1.70

Second, the method may be useful in the measurement of protein. A comparison of this method with the Biuret method (*6*) in the measurement of protein is shown in Table 2. It is evident that the variation between individual proteins with the acetonylacetone method is no greater than it is with the Biuret method and that it would be much less than with methods based on the measurement of a single amino acid (or types of amino acids such as those with 280 mμ absorption, or reactions specific for tryptophan or tyrosine). The sensitivity of the acetonylacetone method is about 10 times greater than that of the Biuret method, as is shown in Table 2. The sensitivity is, however, less than that with the Folin phenol method (*7*) by a factor of 5 to 10.

Third, the method may be useful as a dip reagent in paper chromatography. The most suitable method tried (*8*) resulted in pink to lavender colors with four lambda spots on filter paper of the

Table 2. Comparison of the specificity and sensitivity of the biuret and acetonylacetone methods in the measurement of proteins.

Protein	Optical density/mg at 530 mμ	
	Biuret	Acetonylacetone
Gelatin	0.032	0.375
Egg albumin	0.036	0.265
Lysozyme	0.045	0.406
Gastric mucin	0.039	0.318

following: glycine, arginine, lysine, histamine, tryptamine, ethanolamine, gelatin, egg albumin, lysozyme, and gastric mucin at concentrations of 1 to 2 mg/ml (*9*).

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3. The method used was modified from a standard Elson-Morgan (*1*) reaction as follows: Acetonylacetone reagent was made by adding 1 ml of acetonylacetone to 50 ml of 1*N* Na_2CO_3 and was used in place of the standard acetylacetone reagent. To 1-ml aliquots of the materials to be tested was added 1 ml of the acetonylacetone reagent. After mixing, the tubes were capped and placed in a steam bath for 15 minutes, then cooled. Ethanol (7 ml) and Ehrlich reagent (1 ml) (*1*) were added and then mixed. Readings of optical density were taken after 30 minutes at 530 mμ in a Coleman spectrophotometer.
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8. The following two reagents were prepared immediately before use: acetonylacetone dip (50 ml of acetone and 4 ml of acetonylacetone) and Ehrlich dip (50 ml of 9/1 acetone/HCl (vol./vol.) and 0.5 g of dimethylaminobenzaldehyde). The strip was dipped in the former and heated for 5 minutes at 100°C, and then dipped in the latter and dried in the air.
9. This report is a contribution from the Montana Veterinary Research Laboratory (Montana Experiment Station and Livestock Sanitary Board cooperating), Montana State College, Agricultural Experiment Station, paper No. 441, journal series.

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Mutagenic Effect of Oxygen on Barley Seeds

Abstract. Resting barley seeds stored under oxygen at a pressure of 100 lb/in.^{2} for 4 and 6 weeks exhibit a significant number of chromosome aberrations and mutations. The amount of cytogenetic damage increases with length of storage. The frequencies and types of changes are similar to those induced by 500 r to 1000 r of x-rays.

During experiments (*1*) on the relation of oxygen to the aftereffects of x-irradiation in barley seeds, a distinct mutagenic action of oxygen was found. In 1957 resting seeds of barley (*Hordeum vulgare,* variety Himalaya) were stored for 6 weeks under oxygen and under nitrogen; both gases were at 100-lb/in.^{2} pressure. Other seeds were stored in oxygen and in argon at the same pressure for 4 weeks in 1958. Unstored seeds provided the controls. The moisture content of the seeds was maintained at 8 percent throughout the experiments by the presence of calcium chloride in the storage chambers.

Table 1. Frequencies of chromosome aberrations and seedling chlorophyll mutations induced in seeds of barley by oxygen, nitrogen or argon, at 100-lb/in.^{2} pressure.

Treatment	Chromosome aberrations					Seedling mutations		
		Bridges		Fragments				
	No. of cells	No.	No. per cell	No.	No. per cell	No. of plants	No.	No. per plant
				*Experiment 1**				
Oxygen	300	12	0.040†	28	0.093†	468	20	0.04†
Nitrogen	400	2	0.005	14	0.035	468	4	0.008
Control	400	2	0.005	8	0.020	468	2	0.004
				Experiment 2‡				
Oxygen	600	9	0.015	28	0.047†	803	14	0.017†
Argon	600	2	0.003	12	0.020	774	3	0.004
Control	600	3	0.005	9	0.015	761	1	0.001

* 1957 experiment; 6-wk storage period (summary of four replications). † Significant at the 5-percent level. ‡ 1958 experiment; 4-wk storage period (summary of seven replications).

Dicentric bridges and acentric fragments were scored in the shoot tips of M_1 seeds, and seedling chlorophyll mutations were recorded in the M_2 populations. Details of the storage, cytological, and mutation techniques have been published elsewhere (*2*). Because of the method used in gathering the data, it was theoretically possible to obtain numbers larger than 100 percent; however, in the data presented in this paper the numbers are well below 100 percent; therefore differences between means were tested for significance by the method and tables of Davies (*3*) designed for percentage data.

Significant increases in chromosome aberrations and mutations were found following the oxygen treatment in both experiments (Table 1). Neither the argon nor the nitrogen treatments differed significantly from the control treatments. Thus it appears that pressure alone is not mutagenic.

The difference in the number of chromosome aberrations and mutations between the two experiments is considered to be due to the length of time of storage. In the first experiment the barley seeds were under oxygen for 6 weeks, and in the second experiment for only 4 weeks.

Previous reports have recorded either chromosome aberrations or mutations induced by oxygen in biological material. Conger and Fairchild induced chromosome aberrations in *Tradescantia* pollen by high oxygen pressure (*4*). Since the present study was initiated, high oxygen pressure has been reported to induce mutations in *Escherichia coli* (*5*) and chromosome aberrations in seeds of barley (*6*) and *Crepis capillaris* (*7*).

The frequencies of chromosome aberrations and mutations in oxygen-treated seeds are similar to those induced by 500 r and 1000 r of x-rays (*8*); furthermore, the types of changes induced by both mutagens are similar. These results support the postulate of Gerschman *et al.* (*9*) that a common mechanism may be operating in the biological effects of oxygen and x-irradiation.

It is well known that cytogenetic changes occur in aged seeds, although the cause of these changes is not well understood (*10*). The demonstration of the mutagenic action of oxygen in seeds may aid in understanding this process. Over a prolonged storage period, the atmospheric oxygen may directly or indirectly cause the chromosome breaks and mutations that arise in aged seeds. Furthermore, the results described in the present paper are providing an understanding of the relationship of oxygen to post-x-irradiation damage and to the indirect effects of x-rays in seeds.

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