analyzed for *m*-aminophenol content. The results are given in Table VII.

CONCLUSIONS

1. Condensation reaction between *p*-nitrosobenzoic acid and a variety of amino compounds in sodium bicarbonate solution was studied. *m*-Aminophenol reacted with *p*-nitrosobenzoic acid, producing an intensified, stable orange–yellow dye. The product was isolated and characterized as 3-hydroxyazobenzene-4′-carboxylic acid. In the presence of deactivating groups such as CO_2R, SO_2R, and NO_2, the color reaction of amino compounds was inhibited. Thus, sodium *p*-aminosalicylate, *p*-aminobenzoic acid, benzocaine, *p*-aminosulfonic acid, sulfonamides, *p*-nitroaniline, *etc.*, when tested at concentrations 10,000 times that of *m*-aminophenol, failed to give the color reaction.

2. All aminophenols reacted with *p*-nitrosobenzoic acid, producing discernible colors. The relative rates of color development were approximately in the following decreasing order: $m > o > p$. This color reaction thus can be used for specific identity tests for the three isomeric aminophenols.

3. By using *p*-nitrosobenzoic acid as a specific color-producing agent for *m*-aminophenol, a sensitive and reliable method for the direct determination of *m*-aminophenol in sodium *p*-aminosalicylate or in *p*-aminosalicylic acid was developed. Samples of sodium *p*-aminosalicylate and *o*-aminosalicylic acid containing as little as 0.010% of *m*-aminophenol could be accurately determined.

4. *p*-Nitrosobenzoic acid was used for the first time as a potential colorimetric reagent. Other possible applications of this new reagent are worth investigating in the future.

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Elimination of Choline Interference in Microbiological Assay of Inositol in Pharmaceutical Products

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Abstract □ A method for the assay of inositol in the presence of choline is described which eliminates interference by the latter. By using *Schizosaccharomyces pombe* as the test organism, results comparable to those obtained with *Saccharomyces carlsbergensis* were obtained without the need for separation of the interfering compound.

Keyphrases □ Inositol—microbiological assay in the presence of choline without interference, using *Schizosaccharomyces pombe* as test organism □ *Schizosaccharomyces pombe*—used as test organism to eliminate interference in inositol analysis, compared to *Saccharomyces carlsbergensis* □ Choline—eliminated as interference in nositol microbiological assay

The most widely used method of assay for inositol in the United States, proposed by Atkins *et al.* (1) and Jurist and Foy (2), employs *Saccharomyces carlsbergensis* as the test organism. In pharmaceutical preparations, inositol and choline are often used in combination as lipotropic agents; choline produces significant inhibition of the response of *S. carlsbergensis* to inositol (3).

Various methods have been suggested to eliminate the effect of this interference. It was found that passing dilute aqueous solutions through columns of a cation exchanger[1] produced interference-free eluates. Taylor and McKibbin (3) compensated for the interference by adding choline to the standard tubes. However, both of these procedures involve additional manipulations which are time consuming as well as potential sources of error.

This paper describes a method, routinely used in these laboratories, which permits the direct assay of inositol in the presence of choline without the need for separative or compensatory processes. The assay is based on the method of Norris and Darbre (4).

EXPERIMENTAL

The test organism used was a strain of *Schizosaccharomyces pombe* (ATCC 16491)[2]. The yeast is maintained by monthly transfer on malt agar slants consisting of 10 g. malt extract (Difco), 0.2 g. yeast extract (Difco), and 1.8 g. agar/100 ml. distilled water. Slants are incubated at room temperature for 24 hr. and then stored under refrigeration.

[1] Folin Decalso, The Permutit Co., New York, N. Y..

[2] Obtained from F. W. Norris, Department of Biochemistry, University of Birmingham, Birmingham, England. It is currently available from the American Type Culture Collection, Rockville, MD 20852

Table I—Comparison of Recovery of Inositol from Pharmaceutical Products Using *S. carlsbergensis* and *S. pombe*

Sample	Label Claim, mg./Dose	Inositol Found[a]					
		S. carlsbergensis				*S. pombe*	
		Untreated		Treated			
		mg./Dose	%	mg./Dose	%	mg./Dose	%
Two-piece capsule[b]	83	53.8	64.8	87.5	105.4	85.4	102.9
Soft gelatin capsule[c]	10	6.13	61.3	9.85	98.5	9.20	92.0
Pet supplement (powder)[d]	60	36.9	61.5	64.5	107.5	61.2	102.0
Soft gelatin capsule[e]	40	19.9	49.8	42.0	105.0	40.4	100.8
Pet supplement (tablet)[d]	5	2.44	48.8	4.78	95.6	5.08	101.6
Pet supplement (granule)[d]	5	2.77	55.4	4.53	90.6	5.67	113.4

[a] All values reported are averages of not less than three separate assays. [b] Contains B-complex vitamins, liver, choline, methionine, and inositol. [c] Contains B-complex vitamins, liver, yeast, choline, and inositol. [d] Contains A, D, E, and B-complex vitamins, minerals, liver, yeast, alfalfa, soy, milk, choline, methionine, and inositol. [e] Contains B-complex vitamins, safflower oil, choline, methionine, and inositol.

Basal Medium[3]—The basal medium consisted of the following:

dextrose	40.0 g.	niacin	1.0 mg.
ammonium sulfate	4.0 g.	pyridoxine hydrochloride	1.0 mg.
potassium phosphate, monobasic	3.0 g.	thiamine hydrochloride	1.0 mg.
magnesium sulfate	0.5 g.	D-biotin	0.8 mg.
calcium chloride	0.5 g.	trace elements solution	2.0 ml.
potassium iodide	0.2 mg.	yeast extract	0.1 g.
calcium		lactate buffer	20 ml.
D-pantothenate	1.0 mg.	water	q.s. to 1000 ml.

The trace elements solution used in the basal medium consisted of the following:

boric acid	0.10 g.	ferrous sulfate	0.014 g.
zinc sulfate	0.027 g.	ammonium molybdate	0.019 g.
cupric sulfate	0.029 g.	water	q.s. to 1000 ml.
manganese sulfate	0.030 g.		

Preparation of the yeast extract supplement used in the basal medium as described by Norris and Darbre (4) was too involved for routine control analytical work. Investigation of commercially available yeast extracts indicated that the addition of 100 mg. BBL[4] yeast extract/l. of basal medium gave comparable results. Several other commercial yeast extracts produced very high blanks.

The lactate buffer used in the basal medium is prepared as follows. Concentrated sodium hydroxide solution is added to 250 ml. of syrupy lactic acid until, on diluting 1:100, a pH of 4.8 is obtained. This buffer is then diluted 1:500 for use.

Five to ten liters of basal medium are prepared at one time. The batch is divided into bottles containing approximately 400 ml. each and stored in the deep freeze. Under these conditions, it is stable for at least a year.

Preparation of Samples—Samples are dispersed in distilled water and allowed to stand for several minutes with occasional shaking. Heat may be applied to help solution of inositol from capsules, tablets, *etc.* Samples are diluted in distilled water to an estimated potency of 1 mcg./ml.

Preparation of Inoculum—Growth from a 24–48-hr. culture is transferred to 10 ml. of sterile physiological saline with the aid of a loop. After thorough mixing, the resultant suspension should read about 70% transmission against a saline blank in a colorimeter[5] equipped with a 620-nm. filter (equivalent to an absorbance of 0.078 in a spectrophotometer[6] using 1-cm. cells). If necessary, the concentration of the inoculum is adjusted with cells from another slant or by the addition of saline.

Assay Procedure—A standard curve is prepared in duplicate in 22 × 200-mm. test tubes covering the range of 0–5 mcg./ml. of inositol in 0.5-mcg. increments. Volumes of all tubes are brought to 5 ml. with distilled water, and 5 ml. of basal medium is added to each tube. An additional zero-mcg. tube is prepared for use as an uninoculated blank. Then 1-, 2-, 3-, and 4-ml. aliquots of sample are set up in duplicate. Water and medium are added as previously described. After capping, sample and standard tubes are sterilized in flowing steam for 5 min., cooled in a water bath, and inoculated.

After inoculation, the tubes are incubated for 72 hr. at room temperature on a rotary shaker. At the end of the incubation period, growth is stopped by steaming the run for 5 min.

The colorimeter is set at 100% transmission with the uninoculated blank, and all tubes are read. The averaged standard readings (as percent *T*) are plotted against concentration (in micrograms) on rectangular coordinate paper, and a smooth curve is drawn connecting the points. Sample tube concentrations are read from the curve. Values are converted to a potency per milliliter basis, averaged, and multiplied by the dilution factor to obtain sample potency.

[3] All salts calculated on anhydrous basis.
[4] Baltimore Biological Laboratories.
[5] Evelyn.
[6] Beckman DU.

RESULTS AND DISCUSSION

Table I presents the inositol values obtained on several multivitamin preparations, all of which contain both inositol and choline. Approximately 50–60% of the labeled inositol content was recovered using *S. carlsbergensis* when the choline was not removed. Prior removal of the choline resulted in recoveries approximating label claims.

The proposed method, using *S. pombe*, gave satisfactory inositol recovery without the use of any separative procedures or corrections, making it suitable for use in routine analytical work.

In addition, this organism is not inhibited by relatively high salt concentrations. In the analysis of natural products requiring hydrolysis (HCl), therefore, it is only necessary to neutralize the acid in the hydrolysate instead of removing it by distillation.

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