out the preparation different types of salts were obtained. Sometimes a salt of constant composition was not obtained but what was in all probability a mixture of the different types.

I take this opportunity to express my best thanks to Prof. Rây for his encouragement in carrying out the above investigation.

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TRICRESOL AS A SUBSTITUTE FOR TOLUENE IN ENZYME WORK.[1]

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1. Introduction.

The results of many enzyme experiments are probably worthless because sterile conditions were not maintained by means of a suitable preservative. To overcome this difficulty before beginning a study of the digestive ferments, it was desirable to find a substitute for toluene, the preservative now in general use.

Toluene has several distinct disadvantages: (1) It evaporates readily, the boiling point being 110° to 112°. (2) It often produces a cloudiness which interferes with optical methods, because of its low solubility in water. (3) It clings to the glass in oily drops, introducing errors in volumetric measurement and necessitating the use of a clean pipet each time. (4) Its bactericidal power is uncertain. The deterioration of a casein solution, after standing at room temperature several months, suggested that toluene does not prevent bacterial action, an idea which has been strengthened by a recent article of Benians on "The Resistance of Various Bacteria to the Disinfecting Action of Toluol and the Allied Bodies, Benzol and Xylol."[2]

After trying formaldehyde, which, in strong solutions, caused decoloration with proteins and in weak solutions was ineffective, as were sodium chloride and boric acid, a saturated solution of tricresol was found in the laboratory. According to E. J. Banzhaf,[3] who suggested the use of tricresol in this connection, the late Dr. A. H. Koelker, while working in this laboratory, had determined that a 0.1% tricresol solution did not interfere

[1] Read before the American Society of Biological Chemists, Philadelphia Meeting, Dec., 1913.

[2] T. H. C. Benians, *Z. Chemotherap.*, 1, 28 (1913).

[3] Private communication.

with the action of proteases, using the optical method with peptides as developed by Abderhalden and Koelker. No claim, therefore, is made as to the priority of this idea, but it is believed that no results have heretofore been published on this point.[1]

Tricresol is so well known as a disinfectant that the statement of bacteriologists has been accepted that it is much more powerful germicide than toluene and that 0.1% solution is sufficient to prevent bacterial growth. It is the object of this paper to show that in physical and chemical properties it is more suitable than toluene and that it has little or no injurious effect upon the enzymes.

Tricresol has the following advantages: (1) It is practically non-volatile, the boiling point being 190°. (2) It is soluble in water to the extent of 2%, causing no cloudiness. (3) It runs clean from a pipet.

2. Experimental.

To show the effect of tricresol on the enzymes, a number of experiments have been performed on proteases, (trypsin, erepsin and pepsin) on lipases, a urease, diastases, and on an invertase, using toluene as a standard.

It has been found convenient to use a saturated solution, pipetting off the supernatant liquid. In working with enzymes and proteins, where slight alkalinity or acidity interferes with the action or precipitation, the slightly acid solution was neutralized with $N/10$ sodium hydroxide. One or two series, as examples of each enzyme, will serve to illustrate the procedure and make clear the results.

(*a*) *Trypsin.*[2]—The activity of each of the three proteases, trypsin, erepsin and pepsin was determined by its power to digest protein according to the nephelometric method of Kober.[3]

EXPERIMENT I.

Solutions I.

(*a*) 0.1 g. trypsin in 100 cc. of 0.2% tricresol, allowed to stand at room temperature for 24 hours.

(*b*) 0.1 g. trypsin in 100 cc. of water, and 2 cc. toluene, allowed to stand at room temperature for 24 hours.

Solutions II.

(*a*) 10 cc. of 0.01% trypsin made from solution I*a*, 25 cc. of 0.1% "sodium caseinate" solution[4] 3 cc. 2% tricresol, and made up to 100 cc. with water.

(*b*) 10 cc. of 0.01% trypsin, made from solution I*b*, 25 cc. 0.1% "sodium caseinate" solution, made up to 100 cc. with water to which was added 2 cc. of toluene.

Solutions III.

(*a*) 5 cc. of solution II*a*, 10 cc. of water and 15 cc. of 3% sulfosalicylic acid.

(*b*) 5 cc. of solution II*b*, 10 cc. water and 15 cc. of 3% sulfosalicylic acid.

[1] Koelker, in the last work which he published, used toluene.

[2] Fairchild's "Trypsin."

[3] *J. Biol. Chem.*, **13**, 485 (1913); THIS JOURNAL, **35**, 290, 1586 (1913).

[4] The "sodium caseinate" solutions used in this work were made by dissolving 0.1 g. of casein with 1 cc. of $N/10$ sodium hydroxide and diluting with water.

Solutions III were taken at intervals and nephelometric readings made in comparison with a standard solution containing 5 cc. 0.01% casein solution, 10 cc. of water and 15 cc. of 3% sulfosalicylic acid.

TABLE I.

Preservative.	Time of action. Hrs.	Standard. Mm.	Control. Mm.	15 min. Mm.	30 min. Mm.	60 min. Mm.
Tricresol, 0.2%...	24	15.0	8.0	12.7	22.5	off scale (about 35)
Toluene.....	24	15.0	7.9	11.2	15.5	off scale (about 35)

EXPERIMENT II.

0.5% tricresol solution—other directions were followed as in the preceding experiment.

TABLE II.

Preservative.	Time of action. Hrs.	Standard. Mm.	Control. Mm.	15 min. Mm.	30 min. Mm.	60 min. Mm.	120 min. Mm.
Tricresol, 0.5%...	48	15	6.6	8.2	11.0	16.6	off scale
Toluene.....	48	15	6.7	8.0	10.8	12.1	off scale
Tricresol, 0.5%...	72	15	6.7	8.2	10.9	11.9	off scale
Toluene.....	72	15	7.0	8.0	9.3	11.2	off scale

From the above data it seems that trypsin is not affected by 0.5% tricresol.

(*b*) *Erepsin.*[1]—

EXPERIMENT I.

Solutions I.

(*a*) 0.1 g. of erepsin in 100 cc. of 0.2% tricresol, allowed to stand at room temperature for 24 hours.

(*b*) 0.1 g. of erepsin in 100 cc. of water and 2 cc. of toluene, allowed to stand at room temperature for 24 hours.

Solutions II.

(*a*) 5 cc. of solution I*a*, 5 cc. *N*/10 HCl, 25 cc. 0.1% casein, 3 cc. 2% tricresol, made up to 100 cc. with water.

(*b*) 5 cc. of solution I*b*, 5 cc. *N*/10 HCl, 25 cc. 0.1% casein, 2 cc. of toluene, made up to 100 cc. with water.

Solutions III.

(*a*) 5 cc. of solution II*a*, 10 cc. of water and 15 cc. of 3% sulfosalicylic acid.

(*b*) 5 cc. of solution II*b*, 10 cc. of water and 15 cc. of 3% sulfosalicylic acid.

TABLE III.

Preservative.	Time of action. Hrs.	Standard. Mm.	Control. Mm.	15 min. Mm.	30 min. Mm.	60 min. Mm.	3½ hrs. Mm.
Tricresol, 0.2%...	24	15	4.8	6.7	7.7	13.1	off scale
Toluene.....	24	15	4.8	5.8	6.5	10.6	off scale

EXPERIMENT II.

0.5% tricresol in solution I*a*, and 10 cc. 0.1% erepsin in solution II*a* and II*b*, were the changes made for this experiment.

[1] Johnson and Johnson's Papoid.

TABLE IV.

Preservative.	Time of action. Hrs.	Standard. Mm.	Control. Mm.	15 min. Mm.	30 min. Mm.	60 min. Mm.
Tricresol, 0.5%	24	15	7.1	11.7	17.9	31.5
Toluene	24	15	7.2	11.9	19.6	33.0
Tricresol, 0.5%	48	15	10.7	17.4	23.8	off scale
Toluene	48	15	11.0	21.9	35.0	off scale

The above results show that erepsin does not seem to be affected by 0.2% tricresol during the usual time of incubation (24 hrs.) but the activity is slightly retarded by 0.5% solution in 24 hrs. and that the inhibitory action is more evident on long standing.

(*c*) *Pepsin.*[1]—

EXPERIMENT I.

Solutions I.

(*a*) 0.1 g. of pepsin in 100 cc. of 0.2% tricresol allowed to stand at room temperature for 24 hours.

(*b*) 0.1 g. of pepsin in 100 cc. of water and 2 cc. of toluene allowed to stand at room temperature for 24 hours.

Solutions II.

(*a*) 10 cc. of 0.01% pepsin made from solution I*a*, 25 cc. of 0.1% edestin[2] solution (acid), 3 cc. 0.2% tricresol made up to 100 cc. with water.

(*b*) 10 cc. of 0.01% pepsin made from solution I*b*, 25 cc. 0.1% edestin solution (acid) made up to 100 cc. with water and 2 cc. of toluene added.

Solutions III.

(*a*) 5 cc. of solution II*a*, 10 cc. of water, 15 cc. NaCl solution 1–3.

(*b*) 5 cc. of solution II*b*, 10 cc. of water, 15 cc. NaCl solution 1–3.

Solutions III were compared with a standard solution containing 5 cc. of 0.01% edestin, 10 cc. of water and 15 cc. NaCl solution.

TABLE V.

Preservative.	Time of action. Hrs.	Standard. Mm.	Control. Mm.	15 min. Mm.	30 min. Mm.	60 min. Mm.
Tricresol, 0.2%	24	15	8.0	11.5	27.4	off scale
Toluene	24	15	8.5	11.4	27.4	off scale

EXPERIMENT II.

0.5% tricresol solution in solution I*a* was the only change made for this experiment.

TABLE VI.

Preservative.	Time of action. Hrs.	Standard. Mm.	Control. Mm.	15 min. Mm.	30 min. Mm.
Tricresol, 0.5%	24	15	10.2	15.3	off scale
Toluene	24	15	10.9	16.4	off scale
Tricresol, 0.5%	48	15	10.7	16.4	off scale
Toluene	48	15	10.9	16.7	off scale
Tricresol, 0.5%	72	15	9.0	13.1	26.6
Toluene	72	15	8.6	15.3	off scale

[1] Parke, Davis & Co., Pepsin (scales).

[2] The edestin solutions used in this work were made by dissolving 0.1 g. of edestin in 3 cc. of *N*/10 hydrochloric acid and diluting with water.