counts per cc. but rather as indicators of the relative numbers of viable forms present. They were obtained by plating upon Sabouraud's agar.

TABLE I.
The Effectiveness of Ultrasonic Vibration in Killing Actively Growing Yeasts.

Before Treatment	100,000	28,000	54,000	50,000	21,000	22,800	15,000	125,000	36,500
1 min.	17,000	39,000	2,100	1,800	12,600	22,800	12,500	31,500	4,200
3 "	0	4,300	0	450	9,100	9,100	10,500	3,000	8
5 "	0	110	0	20	3,500	7,700	11,200	360	5
10 "	0	21	0	8	2,200	1,750	5,800	13	5
15 "	0	0	0	0	1,600	lost	3,500	1	5

Microscopic examination of treated culture material revealed much cellular debris and relatively few formed yeast cells. Moreover, transfer from a treated culture to a fresh tube of medium in many instances was followed by no subsequent growth, indicating, therefore, complete sterilization. In other instances, however, these subcultures did show delayed growth.

It appears, therefore, that ultrasonic radiation is an effective agent in killing yeasts. Moreover, the death of the yeast cell may be attended by its disruption.

5888

Prevention of Heat-Coagulation, Mercuric-Precipitation of Proteins, and of Precipitation of Alkaloids by Colloidal Dyes.

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Congo-red and some other colloidal dyes possess the unique action of preventing death in animals injected with surely fatal doses of potent bacterial toxins and drugs.[1] This protective action appears to be mediated through the physical properties of these colloidal dyes. Presumably correlated with this physical protection *in vivo* are some striking protective effects of the colloidal dyes on powerful reactions *in vitro,* such as the heat-coagulation and mercuric precipitation of proteins and the alkaloidal precipitation by Mayer's reagent. As far as I know, these marked protective effects *in vitro* have not

[1] Hanzlik and Butt, *J. Pharm. Exp. Therap.*, 1928, **33**, 260.

been previously described, but they may have considerable scientific importance and practical usefulness.

Heat-coagulation of proteins. Using equal parts of the dyes in 1% strength in 6% dextrose solution, or in distilled water, and of whole serum or saturated solution of dry egg albumin, the following dyes prevented coagulation of the mixtures when boiled: congo red, vital red, trypan red, mercurochrome, rose bengal and sodium fluorescein. Congo red also prevented the heat-coagulation of 50% raw egg white in salt solution. Coagulation did not occur after continuous (half-hour) boiling, and then cooling, of the dye-protein mixtures. The control mixtures, without dyes, were coagulated in less than 1 minute, and after cooling, frequently solidified. The minimum protective amount of congo red for 1 cc. of serum was about 2.5 mg., and certainly protective, was 5 mg. The other dyes were not quantitated, but appeared to be about as efficient as congo red. Acriflavine, which is colloidal according to Hirschfelder and Wright,[2] was protective, but acted somewhat peculiarly: weak and strong solutions precipitated serum, but only the precipitate of strong solutions redissolved in small excess of the solution and such mixtures did not coagulate on boiling. Salyrgan was effective in preventing heat-coagulation of serum. Partially or doubtfully protective were azo blue and trypan blue.

The following colloidal dyes did not prevent heat-coagulation of serum and egg albumin under the same conditions: toluidine blue, neutral red, diamine blue, alkali blue, Chicago blue, aniline blue, induline, nigrosine, new fast green B, night blue, and benzopurpurin. Such colloids as acacia, gelatin, soluble agar, arsenious sulphide, iron, protargentum and indigocarmine did not prevent coagulation of serum. The following crystalloidal dyes did not prevent coagulation of serum and egg albumin; methylene blue, methyl violet, methyl red, phenol red, basic fuchsin, leucosulphato green, sulphato violet, sulphato green, crystal violet (gentian violet).

In all cases, the boiled and protected dye serum mixtures immediately precipitated after the addition of weak solutions of calcium chloride and magnesium sulphate and of saturated sodium chloride, but not of 0.9% sodium chloride and of strong sodium iodide. Under these conditions, the dye was removed from solution with the flocculent precipitate. The salt-effects, but especially those of the weak chloride and the strong iodide, suggest that the physical factor in the protective action of the dyes on heat-coagulation of the

[2] Hirschfelder and Wright, *J. Pharm. Exp. Therap.*, 1930, **39**, 13, 411 and 433.

native proteins is not due to interference with the action of the natural salt (adsorption by the dye), which is essential to heat-coagulation. Rather it would seem that the protective mechanism is in the nature of a surface (peptizing?) action of the dyes on the protein aggregates. In support of this action was the observation that anything which destroyed the physical properties (surface activity) of the dyes impaired or destroyed their protective action, such as the use of a dye in salt solution, the addition of a polyvalent ion or of acid to the dye (congo red), or to the boiled dye-protein mixture, or the addition of mercuric chloride to the latter when hot. Bile salts and caprylic alcohol, which lower surface tension, did not affect the protective action of congo red.

Mercuric chloride precipitation. In the cold, the precipitation of 50% horse-serum and of saturated solution of dry egg-albumin by mercuric chloride, using about 0.05 to 0.1 cc. of 1% mercuric chloride solution to 1 cc. of dye-protein mixture, was prevented by the same dyes which were effective in preventing the heat-coagulation of proteins, while the controls without dyes precipitated immediately. A large excess of mercuric chloride caused some precipitation. It was interesting to observe that the organic mercurial, mercurochrome (2:7 dibromo-4-hydroxy-mercurifluorescein), when first added to serum or egg-albumin, was very effective in preventing the precipitating action of the inorganic compound, mercuric chloride. This is attributed to the colloidal nature of the mercurochrome, recently demonstrated by Hirschfelder and Wright[2] in their studies on the colloid chemistry of antisepsis. In support of the fact that the protective action was associated with the dye-complex of the mercurochrome and not with the mercury ion were the positive protective effects of rose bengal (tetraiododichlorofluorescein) and of sodium fluorescein on the precipitating action of mercuric chloride. As indicated by the results of gelatin-diffusion experiments, it appeared that rose bengal was more colloidal than mercurochrome, and consistent with this property was a high protective efficiency of rose bengal on the heat-coagulation and mercuric-precipitation of the proteins.

Precipitation of alkaloids. Saturated or strong solutions in water of strychnine, morphine, codeine, papaverine, cocaine, atropine, quinine, quinidine, nicotine and curarine, mixed with equal parts of 1% congo red in 6% dextrose solution, did not precipitate after the addition of potassium mercuric iodide (Mayer's reagent). The addition of the dye after preliminary precipitation of the alkaloids left the mixtures turbid or precipitated. Other dyes which prevented

precipitation of a saturated solution of strychnine by Mayer's reagent were: vital red, rose bengal and mercurochrome. The addition of calcium chloride solution to these strychnine-dye mixtures caused immediate precipitation. Trypan blue, trypan red and azo blue did not protect or were indefinite against alkaloidal precipitation. In general, the colloidal dyes which were effective in preventing heat-coagulation and mercuric-precipitation of proteins were also effective in preventing alkaloidal precipitation.

Comment. The full significance of the phenomena here described is not yet apparent, but a few suggestions may be in order. A common physical mechanism for the various protective effects *in vitro* exerted by the colloidal dyes, chemically different, appears to be the basis of these striking phenomena, which suggests that a similar physical mechanism is responsible for their protective effects against the fatal actions of drugs and toxins *in vivo*. Dyes with crystalloidal properties are obviously ineffective, which is also the case with a number of electronegative colloidal dyes and agents tried, possibly because the latter do not consist of sufficiently small aggregates or micellae. Electropositive colloids obviously would not protect. Some of the protective agents here described reputedly act as systemic antiseptics. This encourages the belief that the effects exerted in virtue of the colloidal properties of chemotherapeutic agents may be important in their therapeutic actions. The variety of physically and chemically different agents, useful in the treatment of certain parasitic and infectious diseases, justifies the thought that some common property actuates or supports their therapeutic efficiency. Consider, for example, the variety of agents which are effective in the treatment of syphilis: arsphenamines, mercury, bismuth, tellurium, vanadium, iodide, pyrexia (malaria, sulphur, vaccine), tryparsamide, etc. The mediation of therapeutic effects through the tissues would not be eliminated from the consideration of the colloidal and physico-chemical factors, but should be given greater weight, since it is probable, in fact known to some extent, that crystalloidal chemotherapeutic agents acquire the properties of colloids under biological conditions, and that other agencies affect the tissues and their fluids in the sense of colloidal systems. The implied principle of general protection, through colloidal properties, against the injuries of the virus and products of disease, thus promoting natural defenses, would lack the specific connotations of chemical groups or ions, but could conceivably assist the latter, have broader applications, and explain the results in unrelated conditions.

Conclusions. Certain colloidal dyes, namely, congo red, vital red,

trypan red, mercurochrome, rose bengal and sodium fluorescein, strikingly prevent the heat-coagulation and mercuric-precipitation of horse serum and egg albumin, and also the alkaloidal precipitation by Mayer's reagent. These powerful protective actions *in vitro* appear to be associated with the physical (colloidal) properties of these dyes, in agreement with a similar mechanism of protection exerted by some of these dyes on the actions of potent toxins and drugs *in vivo*. The possible significance of the mechanism of these common protective phenomena, for chemotherapy in general, is briefly discussed.

5889

The Toxicity of Nupercaine.*

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Among the requirements of an ideal local anesthetic agent is prolongation of action. Attempts have been made to meet this requirement, especially in connection with quinine derivatives, but tissue injury at the site of injection has generally precluded clinical use of such agents.[1] Uhlmann[2] introduced the diethyl-ethylene-diamide of butyloxycinchoninic acid (nupercaine, N.N.R.). In Europe this drug came into immediate favor, but a number of deaths were reported[3] following its use. Papers on the toxicity of nupercaine have been published,[4] but since these are not in agreement and did not take up the question of protection, further study seemed indicated.

We began with a 1% solution of nupercaine, but due to tissue irritation, we changed to a 0.5% solution. Rabbits were used and the drug was given subcutaneously or intravenously, with or without

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