obtained from 10 ml of broth is added ½ ml of sterile skim milk. One-tenth ml quantities of this milk suspension are added to sterile, cotton-plugged, straight-sided specimen vials (10 mm × 42 mm). A narrow strip of cellulose tape is placed over the cotton plug to assure its remaining in position. The vials are then placed in a desiccator containing Drierite (anhydrous $CaSO_4$) and lyophilized by holding under vacuum over night. If a pump such as the Cenco Hyvac is used, the contents are quickly frozen and after desiccation yield a white fluffy powder which readily forms a suspension upon the addition of liquid. With a lower vacuum a dark, resinous mass occurs which forms a suspension only after a considerably longer period of time. Although cultures of *L. arabinosus* dried under low vacuum have given results after three months that were comparable to

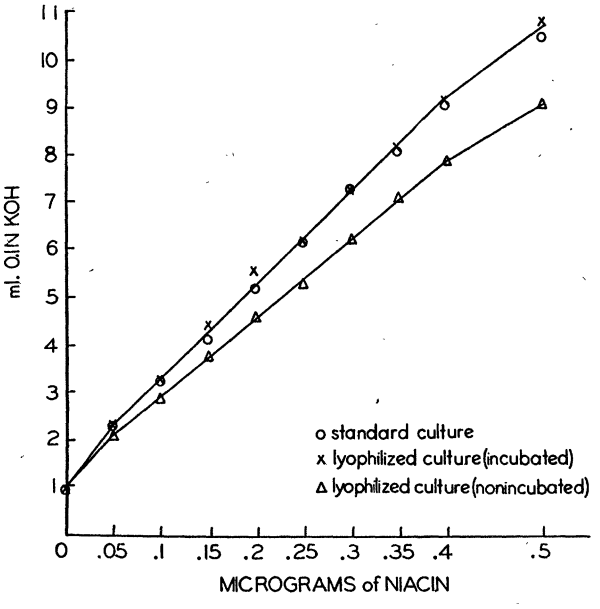

FIG. 1. A comparison of the 72-hour acid production of standard cultures and incubated and nonincubated lyophilized cultures of *Lactobacillus arabinosus* 17–5 in response to added niacin, employing the basal medium of Krehl, Strong and Elvehjem (*Ind. Eng. Chem., Anal. Ed.*, 15: 471, 1943). The lyophilized cultures had been stored two months.

cultures lyophilized by high vacuum, cultures of *L. casei* did not seem as viable when the lower vacuum was used. For best results one should employ high vacuum.

After the contents of the vials are dry (12–18 hours under vacuum), the vials are placed in ¾ inch test-tubes, which are then constricted in an oxygen flame. The tubes are evacuated by means of a high vacuum pump and sealed by rotating in a Fisher burner. The cultures are stored at room temperature away from the light.

For the preparation of the inoculum a desiccated culture is suspended in about one ml of sterile saline or basal medium, and after being stirred by means of a sterile inoculation needle the entire content of the vial is added to 20 ml of diluted basal medium containing the vitamin or amino acid to be assayed (for example, 0.3 μg niacin or riboflavin) and is incubated the customary 24 to 36 hours prior to use. Lyophilized cultures of *L. arabinosus* suspended in 10 ml of saline and used directly as inoculum for assay procedure did not produce as much acid in 72 hours as did the inoculum incubated before use. The response was as linear, however. A comparison of the acid production of standard cultures and of incubated and non-incubated lyophilized cultures of *L. arabinosus* stored two months is shown in Fig. 1.

Further storage studies are in progress and will be reported at a later date. It is felt that the lyophile process as applied to these microbiological assay organisms is saving of time, labor and culture materials, since it obviates the preparation of agar stabs and broths and also the frequent transfer of the cultures necessary to maintain highly viable cells. It also improves the reproducibility of the assay curves in so far as they are influenced by the bacterial cultures.

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ISOLATION OF *TRICHOMONAS VAGINALIS* WITH PENICILLIN[1]

FOLLOWING the initial isolation of a bacteria-free strain of *Trichomonas vaginalis* by Trussell[2] in June, 1939, various investigations of the pathogenicity,[3] physiology[4,5,6,7,8,9,10,11,12] and chemotherapy[13,14] of

[1] Aided by a grant from The Ortho Research Foundation.

[2] Ray E. Trussell, *Jour. Iowa State Med. Soc.*, 30: 66, 1940.

[3] Ray E. Trussell and E. D. Plass, *Am. Jour. Obst. and Gynec.*, 40: 883, 1940.

[4] Garth Johnson, *Proc. Soc. Exp. Biol. and Med.*, 45: 567, 1940.

[5] Alfred B. Kupferberg, *Proc. Soc. Exp. Biol. and Med.*, 45: 220, 1940.

[6] S. H. McNutt and Ray E. Trussell, *Proc. Soc. Exp. Biol. and Med.*, 46: 489, 1941.

[7] Ray E. Trussell and Garth Johnson, *Proc. Soc. Exp. Biol. and Med.*, 47: 176, 1941.

[8] Ray E. Trussell and S. H. McNutt, *Jour. Inf. Dis.*, 69: 18, 1941.

[9] A. B. Kupferberg and Garth Johnson, *Proc. Soc. Exp. Biol. and Med.*, 48: 516, 1941.

this parasite have been carried out in this laboratory. The desirability of obtaining other bacteria-free strains was early realized, but several attempts have uniformly resulted in failure. Consequently, it has been impossible to attack certain problems dealing with antigenic differences, variations in pathogenicity, physiology and growth requirements among strains.

It was recently decided to try the use of penicillin to sterilize new strains. The chances of success were known to be limited because of the reported resistance of many bacterial species to the action of this drug. For this reason, combinations of penicillin with tyrothricin and sulfathiazole offered additional theoretic possibilities.

Seven women with acute or subacute trichomonas vaginitis served as donors of vaginal discharge.[15] Only two of these infestations had received treatment prior to collection of the material. Both had proved resistant to the usual forms of therapy and had persisted for many months. The other five infestations were of unknown duration. The character of the associated bacterial flora was undetermined except for the last donor, who was diabetic and was found to harbor both yeast-like fungi and B-hemolytic streptococci. Each of these women became the source of one experimental strain and the seven strains were collected in the course of six weeks.

The penicillin was obtained from the residual fluid in bottles discarded after clinical administration. The stoppers were treated with 70 per cent. alcohol for ten minutes. Approximately 8 ml of sterile C.P.L.M. medium[13] were introduced by sterile syringe. The contents were mixed and then withdrawn and discharged into a sterile culture tube. The resulting culture fluid was estimated to contain 500 to 1,000 units of penicillin per ml.

Two loops of vaginal discharge obtained by speculum from a given patient were introduced into each of two culture tubes, and the cultures were incubated for sixty hours at 35° to 37° C. Microscopic examination then showed a high trichomonas population.

The first transfers were made into straight C.P.L.M. medium without penicillin. Good cultures were obtained after 72 to 96 hours incubation at 37° C. An additional transfer into the basic medium plus penicillin was tried in one instance. This second culture in the medium with penicillin showed no growth. The exact reason for this is not determined, but it may be significant that this particular sample of penicillin contained adrenalin. Subsequently the contact time with penicillin was limited to 60 hours' incubation without an intervening transfer.

Further transfers were made from the penicillin-treated organisms into C.P.L.M. medium at 40- to 44-hour intervals. In contrast to Trussell's strain, these seven strains of trichomonads tended to grow in clumps when first isolated. This characteristic was found to persist for four to six weeks with gradual diminution. They finally assumed the un-clumped character of Trussell's strain, which had been in culture for six years. Stained preparations revealed a typical morphology.

At the time of the first and second transfers the following media were inoculated to test for the presence of contaminating bacteria: B.B.L. thioglycollate with added dextrose, tomato juice agar (pH 5), blood agar, chocolate agar, nutrient agar, eosin methylene blue agar, selenite F broth, glycerol bile peptone broth, deep meat tubes, deep agar inoculations, 4 per cent. glucose agar, tellurite agar, nutrient broth and blood agar plate in a McIntosh Fildes anaerobic jar. No evidence of bacterial contamination was obtained with any of these media. All subsequent cultures of the seven strains have been tested with the thioglycollate medium. To date, none has given evidence of the presence of either aerobic or anaerobic bacteria.

Combination of penicillin with 100 mg of sulfathiazole per culture tube failed to show any advantage over penicillin alone. A like amount of tyrothricin with penicillin proved lethal to the trichomonads. The use of penicillin alone was accepted as the method of choice.

SUMMARY

Exposure of *Trichomonas vaginalis* in vaginal discharge for 60 hours to 5,000–10,000 units of penicillin in 10.0 ml of a medium containing cysteine (0.15 per cent.), peptone, liver infusion, maltose and human serum was adequate to destroy the associated bacteria. Seven strains were isolated from seven women without a failure. This technic widens the field of investigation by offering a means of obtaining additional strains of bacteria-free *Trichomonas vaginalis* for comparative study.

Efforts to isolate *Trichomonas vaginalis* by such laborious methods as washing, micropipetting and migration have all failed to yield bacteria-free cultures at this and other laboratories. The adherence of bacteria to the trichomonads, which appear to have sticky surfaces, and the relatively slow speed with which these protozoa swim are probably in large

[10] Ray E. Trussell, Mary E. Wilson, *et al.*, *Am. Jour. Obst. and Gynec.*, 44: 292, 1942.

[11] Garth Johnson, *Jour. Parasitol.*, 28: 369, 1942.

[12] Garth Johnson and Margaret H. Trussell, *Proc. Soc. Exp. Biol. and Med.*, 57: 252, 1944.

[13] Garth Johnson and Ray E. Trussell, *Proc. Soc. Exp. Biol. and Med.*, 54: 245, 1943.

[14] Ray E. Trussell and Garth Johnson, *Am. Jour. Obst. and Gynec.*, 48: 215, 1944.

[15] The authors are indebted to Drs. E. D. Plass, W. E. Brown and H. W. Pichette for procuring the specimens.

measure responsible for failure. Further trials by these methods now seem unnecessary.

In the cases reported above the success of the penicillin method may be attributed to several factors. Resistance of *Trichomonas vaginalis* to penicillin in the concentrations reported above, the absence from the specimens of vaginal discharge of bacteria resistant to penicillin in these same concentrations, and the destruction of bacteria adherent to the sticky surfaces of the trichomonads perhaps played a large part in assuring successful isolation of the protozoa.

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DISCUSSION

ADDITION OF CARBON TETRACHLORIDE AND CHLOROFORM TO OLEFINS

FOR many years teachers of elementary organic chemistry have struggled valiantly to combat the persistent tendency of sophomores to write reactions of the following types:

$$RCH=CH_2 + CCl_4 = RCHClCH_2CCl_3$$
$$RCH=CH_2 + CHCl_3 = RCH_2CH_2CCl_3$$

It may, therefore, be of some general interest that these reactions can now be carried out in the laboratory without difficulty. The trick by which this result is accomplished is a simple one. All that is necessary is to add to the olefin an excess of carbon tetrachloride or chloroform and a small amount (about 0.02 mole) of diacetyl or dibenzoyl peroxide, and to heat the reaction mixture.

One or two examples of reactions which have actually been carried out may be of interest.

$C_6H_{13}CH=CH_2 + CCl_4 = C_6H_{13}CHClCH_2CCl_3$
n-octene-1 1,1,1,3-tetrachlorononane

$C_6H_{13}CH=CH_3 + CHCl_3 = C_6H_{13}CH_2CH_2CCl_3$
n-octene-1 1,1,1-trichlorononane

$H_2C=CHCH_2CH_2CH=CH_2 + CCl_4 =$
Diallyl $H_2C=CHCH_2CH_2CHClCH_2CCl_3$
1,1,1,3-tetrachloroheptene-6

$H_2C=CHCH_2CH_2CH=CH_2 + 2CCl_4 =$
Diallyl $Cl_3CCH_2CHClCH_2CH_2CHClCH_2CCCl_3$
1,1,1,3,6,8,8,8-octacholorooctane

$H_2C=CHCH_2CH_2CH=CH_2 + CHCl_3 =$
Diallyl $H_2C=CHCH_2CH_2CH_2CH_2CCl_3$
1,1,1,3,6,8,8,8,-octachlorooctane

In all the reactions cited the yields are good (better than 60 per cent. with carbon tetrachloride); the molecular weights and per cent. chlorine contents of the products agree with the calculated figures. In all instances, some material of higher molecular weight is also formed.

Strange as the reactions cited may appear, the explanation of their mechanisms is not too difficult. Essentially, they are free-radical chain reactions initiated by the free alkyl or aryl radicals generated in the reaction mixture by the decomposition of the organic peroxide. A somewhat schematized version of the steps in the reaction between carbon tetrachloride and the alkylene hydrocarbon $RCH=CH_2$ in the presence of acetyl peroxide is given below. Certain finer details are omitted for the sake of brevity.

$$(1)\ CH_3C(=O)OOC(=O)CH_3 \xrightarrow{\text{Heat}} CH_3\cdot + CO_2 + CH_3COO$$
$$(2)\ CH_3\cdot + CCl_4 \longrightarrow CH_3Cl + Cl_3C\cdot$$
$$(3)\ RCH=CH_2 + Cl_3C\cdot \longrightarrow RCHCH_2CCl_3\ (A)$$
$$(4)\ RCHCH_2CCl_3 + CCl_4 \rightarrow RCHClCH_2CCl_3 + \cdot CCl_3$$

The chain consists in the repetition of the cycle indicated by reactions (3) and (4). The formation of material of higher molecular weight is due to concurrent reactions such as

$$(5)\ RCHCH_2CCl_3 + RCH=CH_2 \rightarrow RCHCH_2CCl_3 \quad (CH_2CHR)$$
$$(6)\ RCHCH_2CCl_3 + CCl_4 \rightarrow RCH_2CH_2CCl_3 \quad (CH_2CHR \rightarrow CH_2CHClR + Cl_3C)$$

For effective repetition of the cycle, the group R in the free radical (A) must be aliphatic.[1] Where R is aromatic (*e.g.*, as in styrene), reactions similar to (5) are favored, and the product consists mostly of higher polymers.

Other additions to olefins which involve an increase in the carbon skeleton are underway in this laboratory. It seems likely that such reactions will be widely applicable.

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"GALL-FLOWER" OF THE FIG, A MISNOMER

IN the Greek literature of 2,000 years ago it is recorded that in Greece and in other parts of the Near East an insect called "psen" or "psenes" inhabited the fruit of the wild fig tree, that the figs from such trees were suspended in domesticated fig trees to insure the setting and maturing of the fruit. It is also recorded that the Greek peasants suspended in their fig trees galls taken from the elm in the belief that the insects that emerged from such galls served the same purpose as those from the wild figs.[1]

[1] Kharasch, Kane and Brown, *Jour. Am. Chem. Soc.*, 63: 526, 1941.