TABLE I.
Pregnancy-Response of Normal Cat's Uterus After Injection of Oestradiol Benzoate[5] and Progesterone.[5]

| Progesterone (total dose) mg. | Av. wt. of cats kg. | No. of cats | Pregnancy-response + | Pregnancy-response — |
|---|---|---|---|---|
| 0 | 1.95 | 4 | 0 | 4 |
| 0.25 | 1.83 | 3 | 0 | 3 |
| 0.50 | 1.95 | 13 | 8 | 5* |
| 0.50 | 2.29 | 4† | 2 | 2 |
| 0.75 | 2.04 | 2 | 1 | 1 |
| 1.00 | 2.25 | 3 | 3 | 0 |

[5] I am indebted to Schering-Kahlbaum A. G., of Berlin, who supplied me with crystalline samples of both hormones.

*Including one experiment in which contraction occasionally occurred if the uterine tone was low.

†Ovariectomized cats.

the anterior pituitary hormone which maintains the corpora lutea. In one cat there was an early pregnancy discovered only at experiment. All the corpora lutea were clearly atrophic; all the embryos were undergoing necrosis. The uterus of this cat usually relaxed after the injection of epinephrine.

9435

## Nutritive Value of Rice Bran Extract for Cultivating Microörganisms.

SAM. C. WONG. (Introduced by C. E. Lim.)

*From the Department of Bacteriology and Immunology, Peiping Union Medical College.*

Rice bran has been implicated by various investigators to contain growth-stimulants. Williams and his associates[1] claimed that pantothenic acid present in the rice-bran extract is chiefly responsible for the growth stimulation of certain strains of yeast. Salle and Dunn[2] working with *Escherichia coli* found that the extent of growth was related directly to the concentration of rice-bran extract added to meat-infusion broth. *Alcaligenes fecalis* did not behave in the same manner. They concluded that the enhancement of growth in the case of *E. coli* was due to the presence of a growth-promoting sub-

[1] Williams, R. J., and Associates, *J. Am. Chem. Soc.*, 1933, **55**, 2912; *Biochem. J.*, 1934, **28**, 1887; *Science*, 1935, **82**, 178.

[2] Salle, A. J., and Dunn, R. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 168.

stance. The presence of vitamin B in rice bran has long been recognized. West and Cruz[3] found that in addition to vitamin B, vitamin E is also present. While the existence of such growth-stimulating substances may well be admitted, it is of interest to determine whether rice-bran extract by itself will serve as an efficient nutrient for cultivating microörganisms.

In our present study it was found that the best extract was prepared by macerating 200 gm. of rice bran with one liter of 12.5% alcohol and incubating at 37°C. for 48 hours with occasional shaking. After centrifugation about 800 cc. of extract was recovered. This was concentrated to about 50 cc. either by vacuum-distillation or by evaporation in a steambath. After cooling, the extract was centrifuged to remove coagulated material, adjusted to pH 7.0 with sodium hydroxide, allowed to stand in the icechest for at least half an hour, centrifuged, made up to 85 cc. and sterilized either by filtering through a Seitz filter or by autoclaving at 10 pounds pressure for 30 minutes. In the latter process of sterilization the extract was adjusted to pH 7.4 before autoclaving. The final extract was stored in the icechest.

Low concentrations of alcohol were better solvents for extraction than high concentrations. The results are shown in Chart 1.

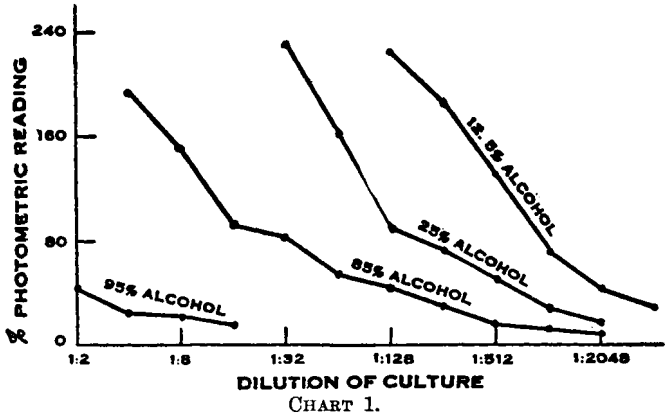


CHART 1.

Growth of *Monilia tropicalis* on rice bran extract prepared by various concentrations of alcohol.

The organism used for the determination of the nutritive substance was *Monilia tropicalis*. This organism was selected after preliminary testing of several species of yeast-like fungi. It was found that all fungi behaved similarly and therefore the results obtained with one were thought to be representative of other similar

[3] West, A. P., and Cruz, A. O., *Philip. J. Science*, 1933, **52**, 1.

fungi. The extracts were diluted with distilled water to various concentrations and inoculations were made with the same suspension of *Monilia tropicalis*. After 48 hours of incubation the extent of turbidity produced by the growth in each dilution of the extract was measured by the Pulfrich photometer[4] using the number 4 opacity-glass as a standard of turbidity. A study of Chart 1 shows that the most nutritive portion was obtained with 12.5% alcohol, the poorest with 95% alcohol. In the former extract, significant growth was observed in the dilution of 1/4098 after 48 hours of incubation. Growth was so profuse at the dilution of 1/64 that a direct reading could not be made. In the latter fraction no growth occurred beyond the dilution of 1/16. It might be mentioned that 95% and 85% alcohol were unsatisfactory solvents for extraction because of the large amount of oil extracted from the rice bran. Likewise, concentrations of 6.25% and 3.12% of alcohol were unsuitable because of heavy bacterial contamination.

A further examination of the chart reveals another interesting point, namely, the quality of the rice-bran extract appears to be related inversely to the concentration of alcohol. In order to show that alcohol plays an important rôle in the extraction the following experiment was carried out. *Monilia tropicalis* was used instead of rice bran because the extracts from the former could be obtained without bacterial contamination, particularly when water or normal saline solution were used as solvents. The organisms obtained were divided into 4 equal portions and treated with (a) dilute ethyl alcohol, (b) boiling water, (c) distilled water, and (d) normal saline. The various extracts in different concentrations in distilled water were inoculated with *Monilia tropicalis* and incubated at 37°C. for 48 hours. The results showed that the amount of nutritive material extracted by boiling water, distilled water, and normal saline was approximately the same while that by the dilute alcohol was many times superior. Significant growth was observed in the dilution of 1/1024 in the alcoholic portion while no growth occurred beyond the dilution of 1/256 in the other portions.

A comparative study of the alcoholic extracts of various vegetable substances such as rice bran, wheat bran, oat bran, freshly grown cultivated mushrooms, and soy bean was made to determine which was the most nutritive. Inoculation of the various dilutions was made with *Monilia tropicalis*. Meat-infusion broth was included as a control. After 24 hours of incubation at 37°C. it was found that the best result was obtained with rice-bran extract while

[4] Kurotchkin, T. J., and Lim, C. E., *Chinese Med. J. Suppl.*, 1936, **1**, 261.

wheat bran, oat bran, mushroom, soy bean, and meat-infusion broth followed in the order named. Growth of *Monilia tropicalis* was not observed beyond the dilution of 1/128 in the case of meat-infusion broth while growth was observed in all others in the dilution of 1/1024.

Comparative studies were made with rice-bran extract and meat-infusion broth with the following cultures: *Escherichia coli, Eberthella typhi, Salmonella paratyphi, Salmonella schottmulleri, Salmonella hirschfeldi, Salmonella enteritidis, Salmonella aertryke, Shigella dysenteriae,* and *Staphylococcus aureus.* The results indicated that rice-bran extract was equal to or better than meat-infusion broth for growing these bacteria. The organisms grown on rice-bran extract were also found to be suitable for serologic agglutination. Of the organisms we have studied, any that can be cultivated on meat-infusion broth can also be grown with equal success on this rice-bran extract.

Chemical analyses of the extract prepared with 12.5% alcohol for total nitrogen by the micro-Kjeldahl method and for reducing substance by the Hagedorn and Jensen[5] method showed that one cc. of the extract contained 5 mg. of nitrogen and 130 mg. of reducing substance. One per cent Bacto-peptone was found to contain 1.1 mg. N per cc. In other words the rice-bran extract is equal to about 5% peptone in nutritive value if the total nitrogen is used as a standard of comparison. It may be stated that the rice-bran extract gave none of the common reactions for protein. The presence of glucose was shown by the osazone test. The whole rice-bran extract was precipitated with 95% alcohol, or anhydrous acetone, or 85% acetone, thereby dividing it into 3 soluble and 3 insoluble fractions. Total nitrogen and reducing substance on these fractions were determined and then the soluble and insoluble fractions in various concentrations were tested with *Monilia tropicalis* and *E. coli* either by themselves or by adding them to a synthetic medium. Since the extent of growth obtained was in accord with the chemical analyses on these 6 fractions, it is sufficient to state that any claim for the stimulation in growth of these microörganisms may be accounted for by the amount of nutritive materials present.

A suitable method is recorded for the extraction of nutritive materials from rice bran. This extract was found to be equal to or better than meat-infusion broth for growing many microörganisms.

[5] Hawk and Bergheim, *Practical Physiological Chemistry,* 19th Edition, 1931.