plaque counting method[4], recently shown in this Institute by Sellers[5] to be possible with the virus of foot-and-mouth disease using monolayers of pig kidney cells.

Although infections with recognized pathogenic agents such as *Rickettsia burneti*, *Brucella abortus*, *Vibrio foetus*, etc., could occur following the use of amniotic membrane or amniotic fluid from a naturally infected fœtus, no evidence of such infection has been observed in this series of cultures prepared from more than twenty-five bovine fœtuses.

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Isolation of β-Dihydroequilin and α-Dihydroequilenin from the Urine of Pregnant Mares

In 1937, Hirschmann and Wintersteiner[1] reported the isolation of 17-dihydroequilenin from the urine of pregnant mares and expressed surprise that no evidence of the presence of dihydroequilin was obtained. (The nomenclature used here is that employed by Carol and his co-workers[3,4,6].) Afterwards, the presence of β-dihydroequilin was detected in material derived from pregnant mares' urine by the dioxane hydrolysis procedure of Grant and Beall[2], but isolation was not effected[3].

We now wish to report the isolation of large quantities of pure β-dihydroequilin from two different concentrates of conjugated œstrogens prepared from pregnant mares' urine collected respectively at mid-pregnancy (approximately the sixth month of pregnancy) and at late pregnancy (approximately the ninth month of pregnancy). It was found that in both instances the proportion of β-dihydroequilin present exceeded the proportion of β-œstradiol. No α-dihydroequilin was found in either concentrate.

The findings of Hirschmann and Wintersteiner[1] were confirmed by the isolation of large amounts of β-dihydroequilenin from both concentrates, but in addition to this α-dihydroequilenin was also obtained. The latter appears to be present in such small proportion that it cannot be considered a major constituent of the naturally occurring œstrogens in pregnant mares' urine.

The concentrates of conjugated œstrogens were prepared by solvent extraction of pregnant mares' urine. Predominantly non-ketonic material was isolated by counter-current distribution methods. This non-ketonic, conjugated material was hydrolysed by heating for 1 hr. at *p*H 1 on the water-bath, freed from ketonic material by a Girard separation, and a phenolic, non-ketonic fraction isolated by extraction into sodium hydroxide. This phenolic, non-ketonic fraction, which was a mixture of œstrogenic diols, was finally separated into its components by the chromatographic methods described by Carol, Haenni and Banes[4] employing sodium hydroxide on 'Celite' (manufactured by the Johns Manville Co.) as the immobile phase and benzene as the mobile phase.

A concentrate of mixed conjugated œstrogens, giving a response by the Kober procedure equivalent to 100 gm. of sodium œstrone sulphate, fractionated by the methods described above, gave a phenolic, non-ketonic fraction which yielded 3 gm. of crystalline β-dihydroequilin (melting point 205–206°), identical with the β-dihydroequilin prepared by Carol, Haenni and Banes[4] by reduction of equilin with aluminium *iso*propoxide, and 1 gm. of β-dihydroequilenin (melting point 216–217°), identical with the material originally isolated by Hirschmann and Wintersteiner[1]; further quantities of β-dihydroequilin could also be isolated from various other side-fractions. Products were characterized by infra-red examination, preparation of derivatives and by analysis.

Throughout laboratory operations care was taken to avoid prolonged exposure of material to strongly acidic conditions. When this precaution was not taken, compound 3 of Hirschmann and Wintersteiner[1] was isolated in addition to β-dihydroequilenin and β-dihydroequilin and was presumably formed by isomerization of the latter in accordance with the findings of Banes, Carol and Haenni[5].

We shall publish this work in greater detail elsewhere.

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Mating Reaction in Yeast

A preliminary study has been made of the mating mechanism of haploid cells of *Saccharomyces cerevisiae*. Three haploid pairs were used: one pair from Dr. C. C. Lindegren and the others derived from two strains of baker's yeast (Lindegren's α = plus; a = minus mating type in Fowell's nomenclature).

Lindegren's discovery[1] that stable haploid clones of two mating types could be derived from the spores of certain strains of baker's yeast made it possible to investigate Winge's suggestion[2] that conjugation is hormone-controlled. When haploid cultures of opposite (plus and minus) mating types are mixed, or cells are paired on an agar film, the cells may put out hyphal ('copulatory') processes; and when these meet, fusion may occur and a dumb-bell-shaped zygote be formed, from which diploid cells may be budded. This sequence of events constitutes the mating reaction, and a cell which puts out a copulatory process may be said to show a mating response.

Experiments in which a plus cell was placed close to a minus cell and allowed to grow on a malt agar film in a moist chamber[3] showed that the mating reaction is probably hormone-controlled, since a