These effects of kidney-antiserum free from antibodies for blood proteins suggest that its nephrotoxic effect is dependent on an antigen-antibody reaction and is not due to the primarily toxic influence of the animal serum or to the presence of antibodies to blood proteins as has been suggested by Pearce[4] and Smadel.[5]

11080 P

Preparation and Diagnostic Value of an Antiserum for Placental Protein.

HAROLD R. COHEN AND VINCENT C. FREDA. (Introduced by William H. Welker.)

From the Departments of Physiological Chemistry and Obstetrics, College of Medicine of the University of Illinois, Chicago, Ill.

This investigation was undertaken first, because a study of the placenta might add to our meager knowledge of tissue antigens and second, a placental antiserum might afford a simple diagnostic test for pregnancy.

There are several reports on antisera for placental proteins. Kintse[1] prepared an antiserum for syncitial material, which contained a specific antiplacental precipitin. Lake[2] isolated nucleoprotein, globulin, albumin, and gelatin from human placenta and by immunization with these produced antisera that reacted with placental proteins and with human serum.

The method of Spinka and Weichselbaum,[3] utilized for the preparation of antisera, makes it possible to obtain directly, without relying on the use of absorptive procedures either *in vivo* or *in vitro,* sera free of antibodies for blood proteins.

Fresh human placentas were frozen and the cord and amniochorion were readily removed. The placentæ were ground in a meat-grinder and then washed with large volumes of chilled saline until snowy white. After further grinding with sand, the fine suspension was washed free of water-soluble protein, adsorbed on aluminium hydroxide cream, and injected intramuscularly into rabbits. After

4 Pearce, R. M., *Univ. Penn. Med. Bull.*, 1903-04, **16**, 217.

5 Smadel, J. E., *J. Exp. Med.*, 1936, **64**, 921.

1 Kintse, *Z. Geburtsch.*, 1912, **72**, 575.

2 Lake, *J. Infect. Dis.*, 1914, **14**, 385.

3 Spinka and Weichselbaum, PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 447.

2 months the serum no longer contained precipitins for human blood-proteins, presumably due to the exhaustion of the blood-proteins from the antigen-depots. Placental autolysates were stratified on the antiserum and reactions were read after one hour at room temperature. A precipitation at the interface appearing after this time was considered a positive reaction. The most potent preparations were obtained after autolyzing the tissue for 8 hours at 37°C.

The organ-specificity of the antiserum was tested with autolysates of 16 normal human organs. Autolysates of ovary, prostate, heart, testis, adrenal, lung, pituitary, pancreas, and breast gave negative reactions. Those of brain, kidney, liver, stomach, uterus, thyroid, and spleen gave positive reactions in varying degrees, those of liver, kidney, and uterus giving the strongest. These results correspond with the findings of other investigations on organ-antisera.

With an antiserum that reacted strongly with placental autolysate, we attempted to demonstrate the presence or absence of placental proteins in 160 samples of blood-sera and urines of pregnant women. Of the known pregnancies tested in the second and third lunar months (4 and 9 cases respectively), a positive reaction occurred with sera in 75% and 78%; with urines in 75% and 33%. In the later months of pregnancy comparable percentages were obtained. The sera of normal non-pregnant women and of males gave negative reactions. Urines that gave positive reactions became negative after standing for a short time and therefore the urines were tested directly after being voided.

Sera of certain gynecological patients, such as fibroid uterus, salpingitis, and cervicitis, gave positive reactions. However, with antiserum from which precipitins for liver and kidney had been removed by *in vitro* absorptive methods, these reactions disappeared. Although strong reactions were obtained with autolysates of placental tissue the reactivity of the absorbed serum had been so diminished that it was no longer capable of detecting the minute amount of placental protein present in the urines and sera of pregnant women. Investigations now under way towards the modification of our technic give promise of production of specific high-titered sera.

Conclusions. A precipitin for placental proteins reacted strongly with autolysates of placental tissue, kidney, liver, and uterus. It reacted with a high percentage of the sera of pregnant women but not with normal sera. Some women with diseased genito-urinary tracts yielded positive sera.