ERRATA

Volume 33, No. 1, pp. 92 and 93, article on Necrotizing Arteriolitis, should read, "buffered solutions had a pH of 7.4."

Article on Gonadotropic Action of Sera, by E. L. Gustus, R. K. Meyer, and J. H. Dingle, indexed page 255, should read page 257.

PROCEEDINGS

OF THE

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

VOL. 33. DECEMBER, 1935. No. 3.

Peiping Section

Peiping Union Medical College, October 23, 1935.

8359 C*

Immunological Potency of Globulin Fraction as Prepared by Methyl Alcohol Precipitation.

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It has been demonstrated that by means of methyl alcohol precipitation fractionation of proteins is possible with sera of dog, ox, sheep and horse and that the protein fractions so obtained can be conveniently converted into dry powder soluble in normal saline.[1] Whether the dry product of such a procedure retains the immune bodies contained therein has not been studied. In this communication is reported the result of experiments on the immunological potency of globulin fraction as prepared by methyl alcohol precipitation. Since human placentas provide a convenient source of pro-

* P represents a preliminary, C a complete manuscript.

[1] Liu, S. C., and Wu, H., *Chinese J. Physiol.*, 1934, **8**, 97.

teins with immune substances,[2] they were utilized to supply the globulin fraction in this study.

From 3 to 5 normal placentas were collected in sterile containers into which a measured amount of 1% solution of sodium chloride was previously placed. The placentas were incised and more sodium chloride solution was added, the total volume of saline being 100 cc. for each placenta. After standing in the refrigerator for 48 hours the mixture was decanted and centrifuged to remove the sediment. Several lots were prepared in this way, and the products were pooled and stored at about —13°C. until the time of precipitation.

The globulin fraction was precipitated by methyl alcohol according to the method of Liu and Wu.[1] The precipitation curve in the case of placental saline extract was previously determined, and was found to assume the same general shape as those of the sera of the horse, dog, sheep and ox.

Thirty-five percent methyl alcohol concentration was taken as the separation point for the globulins in the present preparation. Cold methyl alcohol at about —10°C. was added slowly to the saline extract previously cooled to —1°C. The mixture was allowed to stand at —1°C. for 2 hours. The precipitate was separated in a cold centrifuge and dissolved in normal saline. The saline solution thus obtained was poured into 6 times its volume of ether-ethyl-alcohol (3:7) mixture previously cooled to —25°C. and the mixture was allowed to stand at this temperature for 2 hours. The precipitate was filtered, washed 3 times with ether-alcohol mixture and 3 times with ether, sucked dry and then transferred into an extraction thimble. The whole process was carried out at —25°C. in a cold chamber. The globulin was finally extracted in a Soxhlet apparatus with anhydrous ether over sodium for 24 hours.

The dry powder was kept in a desiccator in an ordinary ice chest until it was dissolved in 1% solution of sodium chloride (5 gm. per 100 cc.) shortly before clinical use. About one-fifth of the powder remained undissolved and it was removed by the centrifugation. A 1% solution of merthiolate was added to the final product so as to make the concentration of the preservative 1:5,000.

Intramuscular and intravenous injections of the extract as prepared by the above described method were first carried out in rabbits. No toxic effect was observed. Then a comparison of immunological potency was made between this extract and that which was prepared

[1] McKhann, C. F., and Chu, F. T., *J. Infect. Dis.*, 1933, **52**, 268.

by ammonium sulphate precipitation,[2] both being produced from the same lot of placental saline extract. The following 2 tests were designed for such a comparison.

1. *Neutralization of Dick toxin.* The potency of the 2 preparations derived from the same lots was first compared with regard to the power of neutralizing Dick toxin. Since the antibody which blanches scarlet fever rashes was previously found to be essentially in the pseudoglobulin fraction of the extract,[2, 3] the concentration of the latter was estimated according to the method of Howe,[4] with the assumption that the proteins precipitated at 14 and 22% of sodium sulphate from the extract are the same substances as those precipitated from the whole serum. The pseudoglobulin concentrations were made equal in any 2 preparations which were to be compared. Intradermal tests were then done on the flexor surface of the forearms in Dick positive persons with the mixture of 0.1 cc. Dick toxin and 0.1 cc. of various dilutions of the extracts, the mixtures being first incubated for 30 minutes at 37°C. In order to make the skin tests comparable, one and the same Dick positive person served as the test subject for the 2 different preparations of the same lot. The result of the tests is shown in Table I. It is clear that the titre for neutralization is about the same for the methyl alcohol and ammonium sulphate preparations of the same lots.

TABLE I.
Neutralization of Dick Toxin.

Dick toxin	Dilution of extract	Lot No. 1		Lot No. 2		Lot No. 3	
		A_1	B_1	A_2	B_2	A_3	B_3
0.1 cc.	1-20, 0.1 cc.	0	0	0	0	0	0
"	1-40, "	0	0	0	0	0	0
"	1-80, "	0	0	0	0	0	0
"	1-160, "	0	0	0	+	0	0
"	1-320, "	+	+	+	+	0	0
"	1-640, "	+	+	+	+	+	+
"	Saline, "	+	+	+	+	+	+

A_1, A_2, A_3 = Methyl alcohol preparations, of globulin in saline solution, with pseudoglobulin 0.92, 2.71 and 2.70 gm. per 100 cc. respectively.
B_1, B_2, B_3 = Ammonium sulphate preparations, diluted to the same pseudoglobulin concentrations as A_1, A_2 and A_3 respectively.
0 = Neutralization.
+ = Redness of more than 1 cm. in any dimension.

2. *Prophylaxis against measles.* In view of the previous experience that measles antibodies seemed to be widely distributed in all the globulin fractions,[3] the concentration of globulin in the saline solution was determined[4] here for the purpose of quantitative com-

[3] McKhann, C. F., Green, A. A., and Coady, H., *J. Pediat.*, 1935, **6**, 603.
[4] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 109.

parison. The solution of lot 4 as prepared by the methyl alcohol method was given intramuscularly in the dose of 0.28 to 0.36 gm. in 5 children, and the sulphate preparation of the same lot, in the dose of 0.36 gm. in 4. All children were known to have been intimately exposed to measles in their brothers or sisters and none had had measles before. The age of the children and the number of days of exposure before the prophylactic injection was given are comparable in the 2 groups. Either modified measles or complete protection was the result in all the cases.

Conclusions. A dry form of placental globulin extract could be prepared by means of methyl alcohol precipitation. When dissolved, this preparation was found to be as potent as the preparation from ammonium sulphate precipitation with regard to neutralization of Dick toxin and prophylaxis against measles.

8360 C

Vaginal Cornification Induced by Swabbing and Its Bearing on the Rat Unit of Estrogenic Substance.

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In the course of a series of assays on one lot of human pregnancy urine extract, employing the method of Coward and Burn,[1] we have encountered findings which were difficult to explain. The recent report of Wade and Doisy[2] directed our attention to the effect of swabbing on the presence of cornified cells in the vaginal smear together with its bearing on the definition of the biological unit of estrogenic substance.

Sexually mature, ovariectomized, albino rats were employed in the experiments. The animals were kept in scrupulously clean cages, each containing 2 or 3 animals. They were fed with a diet in which the supply of vitamin A was adequate. Estrogenic substance was prepared by extracting human pregnancy urine with butyl alcohol by the method described in a previous report.[3] Injections of this extract in olive oil were given by the subcutaneous route.

1 Coward, K. H., and Burn, J. H., *J. Physiol.*, 1927, **63**, 270.

2 Wade, N. J., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 707.

3 Frazier, C. N., and Mu, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 997.