zation of the EEG, development of hypersynchronous activity in the amygdala, and restless, violent behavior attributed by Bokums and Elliott (7) to physostigmine.

Discussion. We have shown that 2-PAM in doses up to 30 mg/kg i.p. does not alter the EEG of cats but that 30–75 mg/kg produce an EEG characteristic of central nervous system depressants that is accompanied by behavioral sedation or tranquilization not progressing to anesthesia within the dose range studied. The depressant action has an anticonvulsant component since 50 mg/kg of 2-PAM prevented induction of convulsions by pentylenetetrazol and 30–75 mg/kg blocked the convulsant action of physostigmine.

The brain of the cat differs from that of the rabbit in its reaction to 2-PAM since Longo *et al.* (5) using doses in the same range as ours found no effect of 2-PAM on the EEG of the normal rabbit brain. In both species, however, these doses antagonized centrally acting cholinergic agents; sarin in the rabbit and physostigmine in the cat. It appears that 2-PAM crosses the blood-brain barrier in cats and rabbits and can achieve concentrations sufficient to antagonize some of the effects of cholinergic and other central nervous stimulants.

Summary. In cats, pralidoxime in doses of 30–75 mg/kg produces sedation and an EEG pattern like that seen after administration of CNS depressants. These doses also prevent convulsions induced by pentylenetetrazol or physostigmine. Apparently significant amounts of pralidoxime cross the blood-brain barrier in cats.

1. Drill's "Pharmacology in Medicine," (J. R. dePalma, ed.), p. 977. McGraw-Hill, New York (1965).

2. Rutland, J. P., Brit. J. Pharmacol. 13, 399 (1958).

3. Firemark, H., Barlow, C. F., and Roth, L. J., J. Pharmacol. Exptl. Therap. 145, 252 (1964).

4. Rosenberg, P., Biochem. Pharmacol. 3, 212 (1960).

5. Longo, V. G., Nachmansohn, D., and Bovet, D., Arch. Int. Pharmacodyn. 123, 282 (1960).

6. Snider, R. S. and Niemer, W. T., "A Stereotoxic Atlas of the Cat Brain," Univ. of Chicago Press, Chicago, Illinois (1961).

7. Bokums, J. A. and Elliott, H. W., Med. Pharmacol. Exptl. Pharmacol. 1, 98 (1968).

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Spermatozoan Decapacitation Factor (DF) in Human Seminal Plasma* (33340)

M. CRAIG PINSKER AND WILLIAM L. WILLIAMS

Department of Biochemistry, University of Georgia, Athens, Georgia 30601

Decapacitation Factor (DF) is a substance demonstrated to be present in the seminal plasma of the rabbit, bull, boar, stallion, monkey, and ram by its ability to prevent fertilization of rabbit ova (1–3). DF, as its name implies, functionally reverses sperm capacitation, the process normally occurring in the female reproductive tract that enables sperm to penetrate ova, a property sperm do not possess at the time of initial deposition in the female (4–7).

The seminal plasmas of the bull and rabbit are potent sources of DF and the sperm of these species require capacitation (4, 5, 7). It appears, therefore, that the presence of DF in seminal plasma is an indication that capacitation occurs in that species. The present paper offers unequivocal evidence that DF occurs in human seminal plasma. Two earlier reports suggested that DF was present in human seminal plasma but fertility was not completely inhibited (1, 2). Brief, prelimi-

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TABLE I. Decapacitation of Rabbit Sperm with a Pellet Obtained by Ultracentrifugation of Human Seminal Plasma.

Treatment of capacitated sperm	No. of oviducts	No. of ova	Ova fertilized (%)
None	2	8	88
Decapacitation attempted with 2 mg pellet/10^6 sperm	2	11	27
None	4	21	67
Decapacitation with 5 mg pellet/10^6 sperm	4	26	0
None	4	9	78
Decapacitation with 7 mg pellet/10^5 sperm	4	22	0

nary accounts of the present work and its confirmation have been recently presented (8, 9).

Materials and Methods. The DF was concentrated from human seminal plasma by ultracentrifugation using the procedure developed for rabbit seminal plasma (10, 11). The reversible inhibition of the fertilizing capacity of rabbit sperm was used as a test for the presence of DF (1). The three essential steps of the assay were the recovery of capacitated sperm from rabbit uteri 11 hr after breeding, incubation of capacitated sperm with the DF preparation for 20 min *in vitro,* and the insemination of capacitated and decapacitated sperm into contralateral oviducts of rabbits 12 hr after an injection of 75 units of HCG or 6 hr after HCG when recapacitation was to be demonstrated.

Results. Sixty-eight ml of human seminal plasma containing 2.80 g of solids, upon ultracentrifugation, yielded a pellet containing 200 mg or 7.2% of the solids. Table I shows that a test level of 2 mg of pellet per 10^5 sperm decreased the percentage of ova fertilized while 5 mg and 7 mg completely prevented fertilization demonstrating the presence of DF in human seminal plasma. To further establish that the rabbit sperm were decapacitated by human seminal plasma as opposed to a nonspecific toxic effect, the *de*capacitated sperm were *re*capacitated by transfer to the oviduct 4 hr before ovulation. This permitted sufficient time for capacitation before the ova become too old to be fertilized. Table II shows that the decapacitated sperm were recapacitated, that the capaciated sperm sample used survived and fertilized ova, and that ejaculated sperm were capacitated under these experimental conditions.

Discussion. Five mg of pellet obtained from 1.5 ml of human seminal plasma was the minimum effective dose for decapacitation. This was significantly greater than the amount used in previous DF assays of human seminal plasma that only lowered the number of ova fertilized instead of completely blocking fertilization (1, 2).

The relatively large amount of seminal plasma solids required for capacitation may indicate either a molecular difference between rabbit and human DF or a markedly lower content of DF in human seminal plasma. Studies on DF from several species have not given an indication of species differences. Although initially DF from bull and rabbit seminal plasma is associated with protein in a different manner, after proteolysis the DF activity chromatographs similarly on two acrylamide gels (3, 11). The results suggest that humans, in common with other primates (8), have DF and therefore very likely a capacitation system. The human capacitation

TABLE II. Recapacitation of Sperm after Decapacitation with Human Seminal Plasma.

Sperm used for insemination into oviducts 4 hr before ovulation	No. of oviducts	No. of ova	Ova fertilized (%)
Sperm decapacitated with 7 mg pellet per 10^6 sperm	7	20	40
Capacitated sperm—same as used for decapacitation	2	7	57
Freshly ejaculated sperm	2	9	55

system may be susceptible to control for contraceptive purposes. This is not likely to be achieved by altering the hormonal state of the female since capacitation in the rabbit uterus is known to be under hormonal control (6, 12, 13).

Summary. Human seminal plasma contains decapacitation factor (DF) as measured by the ability to render rabbit sperm incapable of fertilizing rabbit ova. The presence of DF suggests that capacitation of sperm is required for fertilization in the human. Compared to several other species human seminal plasma has low DF potency. The DF activity sediments in the ultracentrifuge similar to that of the rabbit.

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1. Chang, M. C., Nature **179**, 258 (1957).
2. Dukelow, W. R., Chernoff, H. N., and Williams, W. L., J. Reprod. Fertility **14**, 393 (1967).
3. Williams, W. L., Abney, T. O., Dukelow, W. R., Chernoff, H. N., and Pinsker, M. C., J. Reprod. Fertility, Suppl. **2**, 11 (1967).
4. Austin, C. R., Australian J. Sci. Res. Ser. B **4**, 581 (1951).
5. Chang, M. C., Nature **168**, 697 (1951).
6. Soupart, P., J. Reprod. Fertility, Suppl. **2**, 49 (1967).
7. Trimberger, G. W., Nebraska Agr. Res. Bull. **153**, 3 (1948).
8. Dukelow, W. R. and Chernoff, H. N., Federation Proc. **27**, 567 (1968).
9. Pinsker, M. C., Caster, W. O., Robertson, R. T., and Williams, W. L., Federation Proc. **27**, 567 (1968).
10. Bedford, J. M. and Chang, M. C., Am. J. Physiol. **202**, 179 (1962).
11. Pinsker, M. C. and Williams, W. L., Arch. Biochem. Biophys. **122**, 111 (1967).
12. Chang, M. C., Endocrinology **63**, 619 (1958).
13. Hamner, C. E., Jones, J. P., and Sojka, N. J., Fertility Sterility **19**, 137 (1968).

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Interrelationship of Iron and Manganese Metabolism* (33341)

MARIA DIEZ-EWALD,[1] LEWIS R. WEINTRAUB,[2] AND WILLIAM H. CROSBY

Blood Research Laboratory, New England Medical Center Hospitals and the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111

A relationship between iron and manganese metabolism has been suggested by the observation of the incorporation of manganese into the porphyrin molecule of red cells and the increased gastrointestinal absorption of manganese in iron deficiency (1, 2). The present studies were performed to determine variations in the metabolism of each of these elements induced by deficiency or overload of the other.

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[1] Research Fellow in Hematology, New England Medical Center Hospitals, Boston, Mass. Present address: Instituto de Investigacion Clinica, Maracaibo, Venezuela.

[2] Recipient of U. S. Public Health Service Career Award PHS-AN-31243-01.

Methods. Male albino rats (Wistar strain) weighing 200–250 g were used. The animals were fed a standard rat diet (General Biochemicals, Chagrin Falls, Ohio) except that the iron and manganese content was modified from test to test as stated. Iron absorption was measured following a test dose of 0.5 μCi of ferrous-^{59}Fe citrate per 0.25 mg of ferrous sulfate per 0.5 ml of distilled water, administered through a 17-gauge endoesophageal tube to rats fasted for 16 hr. Whole body radioactivity (0.8 MeV $\longrightarrow\infty$) was measured in a small-animal, whole-body liquid scintillation detector (Packard Armac, LaGrange, Illinois), 3 hr and 7 days after dosing to determine the percentage of the test dose absorbed by the rats.

An isolated intestinal loop technique was utilized to measure manganese absorption be-