Observations on a Precipitin Reaction Between Serum of Patients with Rheumatoid Arthritis and a Preparation (Cohn Fraction II) of Human Gamma Globulin.* (22223)

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Since the observation by Rose *et al.*(1) of agglutination, by sera of many patients with rheumatoid arthritis, of sheep red cells sensitized with nonagglutinating quantities of rabbit antisheep cell serum (amboceptor), a number of modifications of this test system have been described. These fall into two types: those using sheep red cells sensitized with amboceptor against patient's serum or its euglobulin fraction and a system described by Heller in 1954(2). In the latter, sheep red cells, previously treated with tannic acid, are coated with pooled human gamma globulin (Cohn Fraction II). These treated cells are agglutinated when exposed to the serum of patients with rheumatoid arthritis.

In numerous clinical studies(3,4), these test systems have shown a high degree of specificity in patients with indisputable evidence of rheumatoid arthritis. That these tests remain positive when the overt inflammatory reactions have been suppressed by steroid therapy has suggested to us that the circulating factor or factors (rheumatoid factor, R.F.) responsible for these serological reactions may be closely concerned with the mechanisms of this disease. While studying the mechanisms involved in the Heller FII test, it appeared likely that there existed a specificity of interaction between some substance in human pooled gamma globulin and the rheumatoid factor (R.F.), the interaction product of these two on sheep red cells producing visible agglutination. Although the R.F. in the patient's serum has been characterized as to its localization in the Cohn ethanol fractionation system(5), its further identification has been handicapped by the need for a sheep cell system to detect its presence. The following study was undertaken to eliminate the red cell from this system and to precipitate specifically the R.F., thus making it amenable to chemical and immunochemical study.

Methods. Lyophilized pooled human gamma globulin obtained from the American Red Cross dissolved in pH 8 buffered saline to 2.5 g % concentration was absorbed free of naturally occurring antibodies to sheep red cells and heated to 56°C for 30 minutes to inactivate complement. This material was used for coating the sheep red cells for the conventional Heller test and for the precipitation studies. It has been demonstrated previously that addition of extremely small quantities of this gamma globulin to a positively reacting serum from a patient with rheumatoid arthritis results in a negative FII test presumably by the interaction of the gamma globulin added with the R.F. of the patient's serum(2). The sera of subjects studied were complement inactivated and absorbed free of naturally occurring sheep cell antibodies. This treatment of the sera allowed both the precipitin test and the FII test to be done on the same specimen.§ Nitrogen content of gamma globulin solutions was determined by the Kjeldahl method. Euglobulin was prepared by adding equal volumes of serum and distilled water in cellophane bag and dialyzing against distilled water for 48 hours at 4°C. The insoluble fraction (euglobulin) was washed with distilled water until washings were protein-free (Biuret), and redissolved in saline to serum concentration. Initial studies showed that when FII positive serum or its euglobulin fraction was

* Supported in part by grant from Masonic Foundation for Medical Research and Human Welfare.

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§ Precipitation occurs, however, even if the complement of serum and gamma globulin solution is not inactivated.

mixed with varying amounts of gamma globulin, a precipitate resulted. This precipitate formed immediately at the interface of the two components with the larger amounts of gamma globulin when an overlay technic was used. In all studies of precipitate formation, the tubes were first observed for precipitate at the interface, then mixed, allowed to stand at room temperature for 1 hour, placed at 4°C, and finally read by observing the precipitate while shaking the tubes. Aliquots of the supernatants were tested for excess of gamma globulin by the addition of the same serum, for excess rheumatoid factor by the FII test and by the addition of gamma globulin.

Results. Table I shows the result of combining a constant amount of an FII positive serum (0.5 cc of 1:7 dilution in pH 8.0 buffered saline) with varying amounts of gamma globulin. The plus sign indicates the presence of an opalescent gelatinous precipitate. Although quantitative immunochemical studies are not completed, this table suggests an equivalence zone with gamma globulin excess in the supernatant on one side and rheumatoid factor on the other(6).

Table II shows the result of simultaneous dilution of a serum euglobulin and of gamma globulin. Interpreted in the manner of a single antigen-antibody precipitin system, the rapid diminution in the amount of precipitate formed as the euglobulin was diluted (no precipitate at dilution 1:32), while precipitation still occurred with 200-fold dilution of gamma globulin, suggests that the latter or some component of it behaves as the antigen in this system(6).

TABLE I. Precipitation Reaction of Gamma Globulin with Rheumatoid Serum. Serum #145. 0.5 cc of 1:7 buffered saline dilution. FII titer > 56,000. 4°C for 4 days.

mg γ glob. N. in 0.5 cc	Degree of ppt. after 4 days at 4°C	FII test on supernatant	Excess γ globulin in supernatant	Excess R factor in supernatant
.440	4+	0	+	0
.408	"	0	+	0
.377	"	0	+	0
.346	"	0	+	0
.314	"	0	+	0
.283	"	0	+	0
.251	"	0	+	0
.220	"	0	+	0
.188	"	0	+	0
.157	"	0	+	0
.126	"	0	faint pos.	0
.0943	"	0	*Idem*	faint pos.
.0628	"	0	0	*Idem*
.0314	3+	896	0	"

TABLE II. Simultaneous Dilution of Gamma Globulin and Rheumatoid Serum Euglobulin. Dilution of serum euglobulin from a patient with rheumatoid arthritis (FII titer > 56,000) in buffered saline pH 8.0. 4°C for 48 hr. Precipitate measured on a scale of 0 to 4 plus.

mg γ glob. N. in 0.3 cc	Undil.	1:2	1:4	1:8	1:16	1:32	Buffered saline
.381	4+	3+	3+	2+	1+	0	0
.255	"	"	2+	"	"	±	0
.192	"	"	3+	"	2+	1+	0
.096	"	"	2+	"	1+	"	0
.048	3+	2+	"	1+	"	"	0
.0192	2+	"	"	"	"	±	0
.0096	1+	1+	1+	"	"	"	0
.0048	"	"	"	"	"	0	0
.00192	"	"	0	0	0	0	0
.00096	"	0	0	0	0	0	0
Buffered saline	±	0	0	0	0	0	0

The sera of 18 of 21 individuals with the clinical diagnosis of rheumatoid arthritis and a positive FII test showed precipitation under these conditions; no precipitation occurred with the other three. Of 27 individuals with a variety of joint diseases but with negative FII tests, none showed precipitation.

Summary. The precipitin reaction which has been observed will permit study of the 2 factors, one found in serum of patients with rheumatoid arthritis, the other in human gamma globulin, which interact to give a positive FII test (Heller). This reaction eliminates the need for sheep cells and lends itself to investigation by technics of quantitative immunochemistry(6).

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Received December 28, 1955. P.S.E.B.M., 1956, v91.

Growth of Human Epidermoid Carcinomas (Strains KB and HeLa) in Hamsters from Tissue Culture Inocula.* (22224)

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Eagle has reported the isolation of a human epidermoid carcinoma (strain KB) directly from a biopsy specimen(1) in a new medium which satisfies the specific amino acid and vitamin requirements of a mouse fibroblast (strain L) and the HeLa strain of epidermoid carcinoma(2-5). Since strain KB was isolated directly in these culture media without the intervening use of experimental animals, the purpose of the present report is to record its successful transplant from *in vitro* cultures to the cheek pouches of Syrian hamsters. Similar experiments with the HeLa cell also were undertaken, since this tissue culture strain apparently has not yet been studied in the hamster cheek pouch.

Methods. Strain KB, isolated Dec. 21, 1954 and maintained by serial subculture in these new media(1), and strain HeLa(6), similarly maintained in these media for more than a year, were obtained through the courtesy of Dr. Harry Eagle, National Institutes of Health. Both strains had been cultivated in these laboratories for some 4-6 months prior to the present experiments by regular subculture in the medium described by Eagle (2-5), which contains 13 essential amino acids, 7 essential vitamins, glucose and salts, each in the concentration necessary for maximal growth of these 2 cell lines(1-5). The essential serum protein was provided in the present experiments by 10% whole, fresh human serum. The layer of cells growing on the surface of the culture flasks was removed for subculture and for animal experiments by replacing the culture fluid in 8-10-day-old cultures with fresh media containing Difco "1:250" trypsin in a final concentration of 0.125%, and incubating the flask *circa* 5 min at 37°C. The dispersed cells were then sedimented at 5-600 rpm, washed 2 or 3 times with 10-15 ml of culture media to remove the trypsin and resuspended in a convenient volume of complete culture media. The suspensions were shaken gently to disperse the cells, and after enumeration by direct haemocytometer counts, diluted so that the desired number of cells was contained in a volume of 0.1 ml of media. Syrian hamsters, 60-80 g in weight, were prepared for implantation as described by Lutz *et al.*(7). The 0.1 ml of culture media containing the desired inoculum was implanted under the epithelium of the cheek pouch with a 24 gauge needle. Cortisone acetate in subcutaneous doses of 2-3 mg was administered simultaneously with implantation and twice weekly post-implantation(8).

Results. Strain KB. The implantation of 16,000 cells produced a visible nodule in the cheek pouch (Fig. 1a) following a latent period of 56 days. The nodules continued to grow and eventually filled the pouch, presenting the gross appearance of a typical cheek pouch tumor (Fig. 1b). The histology of these tumors† is consistent with that of an

* This investigation was supported in part by research grants (C-2230(C)) and (C-2782) from National Cancer Institute of National Institutes of Health, U. S. Public Health Service.

† We wish to thank Dr. J. Bernstein, Department of Pathology, Children's Medical Center, for histological study of these tumors.