spleen and other parts of the reticuloendothelial system aggravates the injury to the hematopoietic system. A dose ordinarily producing mild injury to the blood forming system evokes, therefore, a severe depression of the blood forming tissues and an increased death rate.

Analysis of energy calculations (energy absorbed per gram of tissue) will probably not be fruitful at this time since the biological effect is dependent not only upon the radiation dosage but upon the radiosensitivity of the tissues. In addition, a great deal of information can be obtained by future experiments. However, these calculations were made and are entered in Table III. It may be seen that the liver and its reticuloendothelial system is very intensively irradiated when Au^{198} singly or Au^{198} and P^{32} combined are given. This would suggest that the liver injury is an important factor in producing the increased death rate.

With doses at the LD_{50} level, the spleen and bone marrow are severely damaged histologically by both colloidal Au^{198} and P^{32}. The liver, however, is only slightly damaged by P^{32} alone while seriously damaged by colloidal Au^{198}.

Summary. Data have been presented on the survival of the albino rat receiving radiation from internally administered P^{32} and colloidal Au^{198}. These isotopes have been administered singly and in combination. The data clearly indicate that P^{32} and colloidal Au^{198} when administered in combination act synergistically in their killing effect. This appears to be related to the simultaneous injury of the reticuloendothelial system and the blood-forming tissues.

We wish to acknowledge the technical assistance of Marvin Berg, Herbert Whanger, Martha Blodgett, and Nancy Shaw.

Received September 18, 1950. P.S.E.B.M., 1951, v76.

Further Studies on Human Hetero-Hemagglutinins for Mouse Erythrocytes.* (18438)

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Natural agglutinins for mouse erythrocytes occurring in human sera have been studied by Gorer(1), and Cohen, Winokur, Kuhns and Figge(2). Gorer(1), using Blood Group A serum, was able to demonstrate antigenic differences in mice by the use of agglutination and adsorption technics. Cohen *et al.*(2) reported that the presence or absence of the "Mo agglutinin," the term they used to designate this hemagglutinin, seemed to divide human sera into two groups independent of their major blood groups. Since only 37 individual sera were studied in the previous work(2) a more extensive investigation was undertaken to obtain more accurate knowledge of the distribution of the Mo agglutinin. This extended investigation has demonstrated that naturally occurring hemagglutinins for mouse erythrocytes are present in all human sera tested. The previous failure to demonstrate this agglutinin in all sera(2) probably resulted from the use of a technic which did not detect positive reactions in sera with low agglutinin titers.

Materials and methods. Four hundred human blood samples were obtained from the Baltimore Rh-Typing Laboratory and the Blood Bank of the University Hospital, Baltimore, Md.† The blood was allowed to clot

* This work was supported by grants from the Anna Fuller Fund and the American Cancer Society, Maryland Division.

1. Gorer, P. A., *J. Gen.*, 1936, v32, 17.

2. Cohen, L., Winokur, G., Kuhns, W. J., Figge, F. H. J., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 548.

and the serum was separated by centrifugation and inactivated by heating in a water bath at 56°C for 30 minutes. Merthiolated serum, 1:5000, was prepared by adding one part 1% solution of sodium ethyl mercuri thiosalicylate‡ (Merthiolate) to 49 parts of serum. Cell suspensions were prepared by placing 2 to 3 drops of blood (obtained from the cut tail of a mouse of the LCSa§ strain) in 2 cc of 0.9% sodium chloride (saline) solution and mixing thoroughly to produce a constant depth of color as judged by the eye. All agglutinations and titers were carried out at room temperature in 10 x 100 mm serological tubes on serum from 1 to 21 days of age. Some of the agglutination tests were made using the method used previously by Cohen *et al.*(2). This agglutination test will be referred to hereafter as the 1:6 serum dilution test, or the 1:6 test. A second agglutination method, hereafter called the 1:2 serum dilution test or the 1:2 test, was performed by placing 2 drops of serum and 2 drops of cell suspension in a tube. These were mixed by shaking and then centrifuged, following which they were read immediately. Agglutination tests were read both macroscopically and microscopically. If definite agglutination could be detected macroscopically, the microscopic examination was omitted and the test called (3+). If many large and small clumps or numerous small clumps of cells could be seen microscopically, and when such clumps could not be defined macroscopically after gentle shaking of the centrifuged specimen, it was read as (2+). If a moderate number of clumps, containing 3 or more cells were found in many fields, it was called (1+). Where evidence of agglutination was indicated in many fields by only 2 or more clumps of only 2 to 4 cells, the reaction was considered to be plus-minus (±). Negative (−) reactions showed no evidence of agglutination. In most cases (−), (±), and (1+) reactions were checked by more than one observer. Titers were determined by placing in tubes, 0.1 cc of serum in serial dilution so that the first tube contained serum undiluted; the second tube, serum diluted 1:2; the third tube, serum diluted 1:4, and so on. All dilutions were made with 0.9% saline. Addition of 0.1 cc of cell suspension made a final volume, in all cases, of 0.2 cc. The serum and cell suspensions were mixed by shaking and then centrifuged. Readings were made as soon as possible following centrifugation. Titers were expressed as the reciprocals of the fraction which indicated the highest serum dilution at which agglutination could be detected. In cases in which (±) reactions were found as the end-point of a titer, the titer was recorded as this number and also the number of the preceding tube. Thus, if a (±) reaction was found in a serum dilution of 1:256, the titer was recorded as 128-256. In agglutination and titer procedures, centrifugation was carried out in an angle (52°) centrifuge at an acceleration range of from 355 to 370 x gravity for 3 minutes. Gorer has pointed out that agglutination of mouse erythrocytes will take place in saline controls if centrifugation is too long or too fast(1). Following preliminary experiments, saline controls were run on all cell suspensions. No agglutination was produced in these controls with the above procedure.

Results. In the preliminary investigation, 53 samples of adult human serum were examined for agglutination by the 1:6 serum dilution test. In each case, 2 samples of serum were run simultaneously. Six of these sera showed enough difference between the 2 identical samples of serum to give a confusing picture, since no clear cut line of demarcation between certain of the positive and negative agglutination reactions could be demonstrated (Table I).

Sera examined by the 1:6 serum dilution test had to contain sufficient antibody to give a positive agglutination reaction in a dilution of 1:6 in order to detect the agglutinins

† We wish to express our appreciation to the Misses E. Jahn and M. Kemp of the Rh Laboratory, and Miss E. Boyle of the University Hospital, for contributing most of the sera used in this work.

‡ Obtained from Eli Lilly Co.

§ LCSa is a strain developed in our laboratory from an A x CBAN cross. The latter mice were progeny of mice obtained from Dr. L. C. Strong in 1941.

TABLE I. Agglutination Variation in 15 Sera Run Simultaneously by the 1:6 Test in Preliminary Experiments.

Serum No.	1st trial	2nd trial	Serum No.	1st trial	2nd trial
R			R		
1	1+	2+	28	2+	±
2	1+	3+	30	2+	—
3	+	—	40	—	±
4	—	—	67	1+	—
8	—	—	73	2+	1+
9	—	—	74	2+	1+
11	±	—	75	3+	1+
24	±	±			

Key to Tables I, II, and III.
— No evidence of agglutination.
± Evidence of agglutination indicated in many fields by only 2 or more clumps of only 2-4 cells.
1+ A moderate number of clumps, containing 3 or more cells found in many microscopic fields.
2+ Many large and/or small clumps seen microscopically when such clumps could not be defined macroscopically, after gentle shaking of centrifuged specimen.
3+ Macroscopic clumping seen.

present. Thus, the limits of discrimination of the 1:6 test were too near the titer end points of many of the sera. The 1:2 test was developed, therefore, so that agglutinins present only in sufficient quantities to give a titer of 2 could be detected, and results obtained with the 2 tests were compared. Three hundred and forty-seven samples of blood representing the major blood groups, both Rh positive and Rh negative, obtained from white and Negro individuals of both sexes were examined. In 38 instances, 2 samples were obtained from the same individual. A total of 392 examinations, using both the 1:6 and 1:2 test, were performed on these sera. As determined by the 1:6 test, 16 sera were negative, 5 gave (±) reactions, 8 gave (1+) reactions, 54 gave (2+) reactions, and 264 gave (3+) reactions. There were no negative results with the 1:2 test, only 1 (±) reading,‖ 4 (1+) reactions, 37 (2+) reactions and 305 (3+) reactions (Table II). Of the 26 sera on which more than one determination was made, results obtained with the 1:2 test varied sufficiently in only one case to make it doubtful whether agglutinins were present or not,‖ while 8 sera gave confusing results with the 1:6 test. In cases where more than one sample of blood was obtained from one individual, comparison of the results of these two samples yielded similar information. In the 392 examinations performed, the 1:2 test gave weaker agglutination reactions than the 1:6 serum dilution test in only 13 cases.

‖ This serum was subsequently checked and gave a positive reaction with the 1:2 test although the 1:6 test had a persistent negative reaction.

The Mo agglutinin titer of 29 sera was determined (Table III). Of 10 sera having titers of 8 or below, only 2 gave indication of agglutination in the 1:6 test. Of the 19 sera having titers of 8-16 or above, 4 had reactions in the 1:6 test which were not clearly positive. Only 2 of these sera had titers as high as 16. This demonstrated, further, that the 1:6 test did not detect agglutinins in sera with low titers.

TABLE II. Comparison of Agglutination Reactions Obtained with 347 Sera Using the 1:2 and 1:6 Test.

	No. of sera showing each degree of agglutination					
Test	—	±	1+	2+	3+	Total
1:6	16	5	8	54	256	347
1:2	0	1*	4	37	305	347

* This serum was subsequently checked and gave a positive reaction with the 1:2 test, although the 1:6 test had a persistent negative reaction.

TABLE III. Titers of Mo Agglutinin Content Compared with Results Obtained in the 1:6 and 1:2 Test.

Serum No.	1:6 test	1:2 test	Titer	Serum No.	1:6 test	1:2 test	Titer
B				B			
301	3+	3+	256	601	2+	2+	16
298	3+	3+	256	272	2+	3+	8-16
585	3+	3+	256	592	—	3+	8-16
583	3+	3+	256	590	—	3+	8-16
584	2+	3+	128-256	598	2+	2+	8
305	3+	3+	128	594	—	3+	8
600	2+	2+	64-128	593	—	2+	8
307	2+	2+	32- 64	288	2+	3+	4-8
278	3+	3+	32- 64	591	—	3+	4
587	2+	3+	32	268*	—	±	4
597	2+	2+	32	602	—	3+	2-4
296	2+	3+	16- 32	596	—	3+	2-4
261	3+	3+	16	279	—	1+	2
297	±	3+	16	595	—	3+	<2
300	±	3+	16				

* This serum was subsequently checked and gave a positive reaction with the 1:2 test, although the 1:6 test had a persistent negative reaction.

In the previous work(2), sera which were tested for Mo agglutinins were preserved with Merthiolate. To determine whether Merthiolate had a destructive effect on this agglutinin, 16 sera were collected and within 24 hours were inactivated and divided into 2 portions, to one of which Merthiolate was added as described previously. These sera were then stored in a refrigerator for periods ranging from 6 to 19 days following which the merthiolated and unmerthiolated sera were tested simultaneously. The difference between titers of serum with and without Merthiolate never varied more than one tube. In 5 cases, the titer of merthiolated serum was ½ tube lower than the serum without Merthiolate. In one case, the titer of merthiolated serum was one tube higher and in another case ½ tube higher than the serum without Merthiolate.

Discussion of results. The Mo agglutinin, a naturally occurring human hetero-hemagglutinin for mouse erythrocytes, was found in all the 400 sera tested in this investigation. The previously reported(2) failure to detect agglutinins in sera of some human subjects was undoubtedly due to the fact that the method did not detect agglutinins in sera with low titers. Waller(3) has shown that Merthiolate, in small concentrations, inhibits the action of anti-Rh agglutinins. If this were true of the Mo agglutinin, it would constitute additional reason for the large number of sera which were reported as not containing the agglutinin in the previous investigation. Results obtained in the merthiolated series of sera seemed to indicate that there might be a small degree of inactivation of the Mo agglutinin by Merthiolate. However, this inactivation was not of sufficient degree to account for the results obtained previously. Age, sex, color or major blood groups were not related to the degree of agglutination exhibited by the sera tested. Gorer(1) has used what, in all probability, is this agglutinin in detecting antigenic similarities and differences in various strains of mice. Thus this agglutinin was used to detect what he has called Antigen III. The question of whether the Mo agglutinin may have some significance comparable to the heterophilic antibody found in infectious mononucleosis should be investigated.

Summary. The Mo agglutinin (a human agglutinin for mouse erythrocytes) was found to be present in 400 human sera tested. The failure of previous attempts to demonstrate the agglutinin in all sera was probably due to the fact that the method used was not sufficiently sensitive. Merthiolate has little or no inactivating effect on the Mo agglutinin. The significance of the variation in amount of Mo antibody in human sera has not been determined.

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Received October 10, 1950. P.S.E.B.M., 1951, v76.

Neurotropic Viruses in Extraneural Tissues. III. MM Virus in Human and Murine Testicular Tissues.* (18439)

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In previous papers of this series(1,2), MM virus has been shown to multiply in the following tissues of hamsters and/or mice: soft tissue of the hind foot, skeletal muscle, skin, pharyngeal wall, urinary bladder wall, and embryonic tissues. All of the specimens of tissue in which virus was shown to multiply contained either smooth or striated muscle.

* Aided by a grant from the National Foundation for Infantile Paralysis.

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2. Chambers, V. C., Smith, W. M., and Evans, C. A., *J. Immunol.*, 1950, v65, No. 6.