and time lapse before death in series B, where an immediate injection of penicillin was given, and in series E and F where administration of penicillin was delayed for 3 and 6 hours respectively, may be explained by the fact that it is generally acknowledged that penicillin is most effective against sensitive microorganisms during the period of active growth and multiplication; therefore the efficacy of penicillin administered after 3- or 6-hour lapses may indicate that those times allow germination of spores[8] and the vegetative cells are then inhibited by the concentration of penicillin available. Since Penicillin G Procaine in oil is reported to maintain concentrations at effective therapeutic levels for 96 hours, the longer time lapse before symptoms appear may be accounted for if it is only after this level has dropped that a lethal toxin is produced by survivors.

Higher mortalities were observed in series C and G. When 150 units of penicillin was administered with no delay but in the leg opposite the necrosis, 80% of the mice died (Table I, Series C). When the penicillin was injected into the necrotic leg after a 24-hour delay, 52.6% of the mice died (Table I, series G). The indication in series C is that (1) a sufficient amount of penicillin does not reach the necrotic area to inhibit growth and production of toxin by *Cl. tetani*, with resultant death of the animal, or (2) that organisms produce a lethal dose of toxin before being inhibited, with this latter possibility being especially applicable to series G. The rate at which penicillin is released and the rate and extent of penetration into necrotic areas are probably the significant factors. The similarity of series C and G to series A (Table I) in the short time lapse before death indicates that toxin production had taken place rapidly, and if series G is compared with series F this observation is further verified since the additional 18 hours time lapse before penicillin was administered caused a 30% increase in mortality.

Conclusions. Results indicate that penicillin-procaine G in sesame oil and 2% aluminum monostearate is of significant value prophylactically in lowering the mortality of mice experimentally infected with a lethal dose of detoxified spores of *Clostridium tetani*. In addition to decreasing the mortality, it retards the development of symptoms and resulting deaths. Injected into the necrotic areas, it is more effective than the same unitage injected at an uninfected site. Whether this is caused by insufficient penetration of the drug due to the presence of necrotic tissue or to interference by the calcium chloride has not been determined.

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Further Observations on Isosensitization to the Rh Factor.

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The purpose of this report is to describe the results of experiments on Rh sensitization in man. The findings are of significance in relation to the pathogenesis of intragroup hemolytic transfusion reactions,[1,2] and the pathogenesis of erythroblastosis fetalis,[3] as well as the practical problem of producing Rh testing sera.[4]

The subjects used in these experiments were 47 adult male individuals. Each individual was subjected to a series of injections of group

1 Wiener, A. S., and Peters, H. R., *Ann. Int. Med.*, 1940, **13**, 2306.

2 Unger, L. J., and Wiener, A. S., *Am. J. Clin. Path.*, 1945, **15**, 280.

3 Wiener, A. S., *Am. J. Dis. Child.*, 1946, **71**, 14.

4 Wiener, A. S., and Gordon, E. B. S., *Am. J. Clin. Path.*, 1947, **17**, 67.

TABLE I.
Observations on Rh Sensitization in Males.

No. of inj.	No. of subjects	Frequency of Rh antibody formation		% of total series first showing Rh antibodies at inj. in question	Cumulative total (%)	
		No.	%		Sensitized	Not sensitized
2	47	18*	38.3	38.3	38.3	61.7
3	23	6	26.1	26.1 × 0.617 = 16.1	54.4	45.6
4	15	3	20.0	20.0 × 0.456 = 9.1	63.5	36.5
5	10	1	10.0	10.0 × 0.365 = 3.7	67.2	32.8
6	3	1	33.3	33.3 × 0.328 = 10.9	78.1	21.9

* One subject produced Rh antibodies after the very first injection. In this case a history was elicited of several blood transfusions received many years previously during treatment for osteomyelitis.

O, Rh_0-positive blood, each dose consisting of 2 to 4 cc of a 50% suspension of whole blood in sodium citrate solution. The injections were given at intervals of 3 to 4 months. All the injections were given intravenously, except that occasionally part of the blood was deposited into or under the skin. Tests for Rh antibodies were made in the usual manner[5,6] by the agglutination and albumin-plasma conglutination methods. The antibody tests were carried out 10 to 14 days after each injection, except that in most cases no tests were done after the very first injection. According to our previous observations,[2,4] as a rule a minimum of 2 injections, spaced 3 to 4 months apart, are needed to induce the formation of Rh antibodies, the first injection acting as a primer and the second or subsequent injection stimulating the production of specific antibodies.

The results are summarized in Table I. It will be seen that after the second injection, 18 of the 47 subjects, or 38.3%, had demonstrable antibodies in their sera. One of these 18 subjects had showed antibodies after the very first injection, but after careful questioning a history was elicited of previous blood transfusions many years before when this individual had been treated for osteomyelitis. Of the 29 subjects who failed to respond to the second injection, 23 returned for a third injection, and 6 of these or 26.1% then showed antibodies. If we assume that the 6 individuals who did not return for the third injection had an equal chance of becoming sensitized, then this indicates that the third injection would sensitize 26.1% of the 61.7% of the nonsensitized individuals remaining after the second injection, or 16.1% of the total number injected. By adding this 16.1% to the 38.3% representing the individuals sensitized by the second injection, we find that fully 54.4% of the Rh-negative individuals became sensitized after a course of 3 properly spaced injections of Rh-positive blood. Of the 17 individuals who failed to respond to the third injection, 15 returned for a fourth injection, and of these 3 individuals or 20% became sensitized. As shown in the table, this raises to 63.5% the total incidence of sensitization after a course of 4 injections; and similarly, the percentage of sensitized individuals is raised to 67.2% by a fifth injection of Rh-positive blood. Unfortunately, only 3 subjects returned for the sixth injection, of whom one became sensitized. Obviously, this result has a large statistical error and should therefore be discounted. Judging from the results of the third, fourth, and fifth injections, it would appear as if the percentage of sensitized individuals was approaching a limit somewhere between 70 to 80%; while if the results of the sixth injection could be taken literally, it would seem that all the injected individuals would eventually become sensitized following a sufficient number of injections of blood. This question can obviously only be settled by experiments on a larger series of individuals, over a longer course of injections.

In a parallel series of experiments, a number of type Rh_1Rh_1 individuals was subjected

[5] Wiener, A. S., *Am. J. Clin. Path.*, 1946, **16**, 477.

[6] Wiener, A. S., and Hurst, J. G., *Exp. Med. and Surg.*, 1947, **5**, 285.

to a series of injections of type rh blood in an attempt to induce sensitization to the factor hr′ or Hr_0, or both. Nineteen of these individuals received 2 injections; of these 13 received a third injection, 8 received a fourth injection, 5 received a fifth injection, while 4 received a sixth injection. Not one of the individuals of this series showed any Hr antibodies at any time. This, despite the smaller number of individuals injected, is in sharp contrast to our experiences with sensitization against Rh_0 factor. Moreover, these results conform with the conclusions based on clinical observations that the Hr factors are much weaker antigens than the Rh factors.[7]

Summary. The results of experiments on sensitization of male type rh individuals by intravenous injections of Rh_0-positive blood at intervals of 3 to 4 months are described. Of 47 subjects receiving 2 injections, 38.3% became sensitized; after 3 injections, the incidence of sensitization rose to 54.4%; after 4 injections, the percentage rose to 63.5%, while after 5 injections the incidence of sensitization was 67.2%. Because of the small number of individuals receiving more than 5 injections, it is not possible to state with certainty whether the incidence of sensitization approaches a maximum limit somewhere between 70 and 80% of individuals injected, or whether all individuals eventually would become sensitized providing they are given a sufficient number of injections. In any event, it is obvious that the deliberate injection of Rh-positive blood is a potent means of sensitizing Rh-negative individuals.

In contrast to these results, not one among 19 type Rh_1Rh_1 individuals receiving repeated injections of type rh blood became sensitized to the Hr factors. These results confirm the conclusion based on clinical observations that the Hr factors are much weaker antigens than the Rh factors.

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[7] Wiener, A. S., *J. Lab. and Clin. Med.*, 1948, **33**, 985.

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Glycolysis in *Plasmodium gallinaceum.**

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For many years a practical method of cultivating the malaria plasmodia has been sought, and recently one has been devised by Ball and his colleagues[1,2] for the erythrocytic stages of *Plasmodium knowlesi*, a parasite of monkeys. Monkeys, however, are expensive and much less easily maintained in the laboratory than birds, and for this reason it is important that a good culture technic be devised for the avian plasmodia,‡ which are equally suited for the screening of antimalarial drugs and for other types of research in malaria. It

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[1] Ball, E. G., Anfinsen, C. B., Geiman, Q. M., McKee, R. W., and Ormsbee, R. A., *Science*, 1945, **101**, 542.

[2] Geiman, Q. M., Anfinsen, C. B., McKee, R. W., Ormsbee, R. A., and Ball, E. G., *J. Exp. Med.*, 1946, **84**, 583.

‡ Hawking[3] has devised a tissue culture technic for growing the exoerythrocytic stages of several species of avian plasmodia.

[3] Hawking, F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1945, **39**, 245.