2. Norman, A., Sjövall, J., *J. Biol. Chem.*, 1958, v233, 872.
3. Gustafsson, B. E., Bergström, S., Lindstedt, S., Norman, A., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 467.
4. Norman, A., *Acta Physiol. Scand.*, 1955, v33, 99.
5. Norman, A., Grubb, R., *Acta Pathol. et Microbiol. Scand.*, 1955, v36, 537.
6. Norman, A., *Arkiv f. kemi*, 1955, v8, 331.
7. Sjövall, J., *Acta Chem. Scand.*, 1954, v8, 339.
8. ———, *ibid.*, 1952, v6, 1552.
9. Norman, A., *ibid.*, 1953, v7, 1413.

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Plasma Levels of Various Blood Clotting Factors in Germfree Rats.* (29604)

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The blood clotting factors prothrombin (factor I), proconvertin (factor VII), antihemophilic B factor (factor IX) and Stuart-Prower factor (factor X) depend on vit. K for their production and are all produced in the liver. Fibrinogen (factor II), proaccelerin (factor V) and antihemophilic A factor, (factor VIII) may, however, mainly be produced elsewhere in the body. It has been shown that the antihemophilic A factor level in plasma may be considerably raised by injections of adrenalin(1), or after muscular exercise(2,3,4). Fever-producing vaccine injections(5) or unspecific fever induction(6), cause increases in the plasma levels of antihemophilic A factor, proaccelerin and fibrinogen. This increase may indicate that their production is related to the reticulo-endothelial system(5,6). The theory is supported by the observation that patients with diseases of the reticulo-endothelial system, for example Hodgkin's disease, may have very high plasma levels of antihemophilic A factor(7).

These data suggested that it would be of interest to analyse the plasma levels of the blood clotting factors in germfree animals, in which part of the lymphatic system is poorly developed(8,9,10,11,12,13).

Methods and materials. Animals. Seven germfree rats of the Swedish strain from the Department of Germfree Research at Karolinska Institutet were used. These animals were housed in Gustafsson's isolators, and reared according to Gustafsson's methods(9, 14). The 7 control animals, of comparable weight and age, were of the same strain and reared in the same institute as the germfree rats. Each rat received 200 μg vitamin K_1 per day in its food.

Blood collection. Blood was obtained from ether-anesthetized animals by puncture of the right heart ventricle after thoracotomy. 2.7 ml blood was drawn into a plastic syringe containing 0.3 ml of a 3.1% Na-citrate solution ($2H_2O$), transferred to a chilled, paraffinized glass tube and centrifuged for 20 minutes. The plasma was kept deep frozen in a paraffinized glass tube until tested.

Blood clotting tests.

Antihemophilic A factor was measured with the thromboplastin generation test of Pool and Robinson(15).

Fibrinogen was measured spectrophotometrically by the method of Jacobsson(16) with some minor modifications(17).

Prothrombin was measured with the one-stage method using Russel's viper venom in cephalin as reagent(18), modified so as to be insensitive to the plasma content of Stuart-Prower factor(19).

Proaccelerin was measured according to Stormorken(20).

Proconvertin and *antihemophilic B factor* were measured in one-stage systems using

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TABLE I. Plasma Levels of Blood Clotting Factors.

Blood clotting factor or test	Germfree animals		Control animals		P values of differences between 2 groups
	Range	Mean	Range	Mean	
Fibrinogen (I)	150–234 mg%	180 mg%	144–224 mg%	167.4 mg%	$.4 < p < .5$
Prothrombin (II)	66–120%	90.6%	86–135%	99.9%	
Proaccelerin (V)	68–126%	100.1%	86–117%	98.1%	
Proconvertin (VII)	90–120%	105.4%	84–125%	107.3%	
Antihemophilic factor A (VIII)	78–125%	98.7%	101–115%	109.6%	$.1 < p < .2$
" factor B (IX)	74–185%	114.9%	68–140%	95.3%	$.2 < p < .3$
Stuart-Prower factor (X)	74–206%	135.6%	64–105%	85.7%	$.02 < p < .05$
Thromboplastin time	16.8–21.4"	18.7"	16.9–22.4"	19.4"	
Cephalin time	26.7–33.2"	29.9"	24.5–38.2"	31.5"	
Proaccelerin-accelerin capacity	6.3–11.7	8.5	7.6–11.4	9.6	
Activation capacity	1.0– 2.1	1.67	1.4– 2.4	1.97	

For factors II–X, values are given as per cent of mean plasma level in a large group of normal, conventional rats.

plasma from patients with congenital deficiencies of these factors as substrate reagents(19,21).

Stuart-Prower factor was measured in a one-stage system based on a plasma reagent where this factor had been specifically removed. The reagent was kindly given us by P. A. Owren(22).

Thromboplastin time and *cephalin time* tests were performed as described by Waaler (19).

Proaccelerin-accelerin capacity expresses the relation between the activity of plasma as measured in the proaccelerin system after and before addition of 0.5 I.U. of thrombin to 0.8 ml of plasma(23).

Activation capacity expresses the increase in plasma coagulability after contact with a large silicate surface(19).

Results and discussion. The main results are given in Table I. The plasma levels of the blood clotting factors tested were within normal range in all animals of both groups. It is of special interest that the plasma levels of fibrinogen, proaccelerin and antihemophillic A factor showed no significant differences between the two groups of animals, since these clotting factors are probably produced in the reticulo-endothelial system. The mean antihemophilic A factor level was somewhat lower in the group of germfree rats but was not significantly different from the mean level in the control group. The ability of the plasmas to form accelerin on thrombin addition was similar in the two groups of animals, indicating a qualitative as well as a quantitative similarity in their proaccelerin.

Plasma levels of Stuart-Prower factor and antihemophilic B factor were somewhat higher in the germfree rats than in the control rats with a statistically significant difference (at the 5% levels) for the former factor. The difference might result from a better utilization of the large daily dose of vit. K in the germfree rats.

The first phase of "intrinsic" blood coagulation involves Hageman factor (factor XII) and antihemophilic C factor (factor XI), which we have been unable to measure directly. The similarities between the two groups of animals in cephalin time and in activation capacities indicate, however, that this first phase of "intrinsic" blood coagulation is normal in the germfree rats. The relatively small plasma "activation capacity" in both groups of animals might be due to the plasmas having been deep frozen and then thawed.

These investigations do not exclude the reticulo-endothelial system as production site for fibrinogen, proaccelerin and antihemophilic A factor. The tissue which is involved in such production must, however, be sufficiently well developed in germfree rats to maintain normal plasma levels of the factors in question.

Summary. A group of 7 germfree rats had a somewhat lower mean plasma level of antihemophilic A factor (factor VIII) than 7 control rats. The difference was not statis-

tically significant. There were no differences between the 2 groups of animals in their mean plasma levels of fibrinogen (factor I) and proaccelerin (factor V), which are the 2 other blood clotting factors possibly produced in the reticulo-endothelial system. All rats received the same daily dose of vit. K_1 in their food. The germfree rats, however, showed a higher mean plasma level of antihemophilic B factor (factor IX) and Stuart-Prower factor (factor X) than the control rats, the difference in the latter factor being statistically significant at the 5% level. No other differences were detected between the 2 groups of animals as far as known blood clotting factors are concerned.

1. Ingram, G. I. C., *J. Physiol.*, 1961, v156, 217.
2. Rizza, C. R., *ibid.*, 1961, v156, 128.
3. Egeberg, C., *Scand. J. Clin. Lab. Invest.*, 1963, v15, 8.
4. ———, *ibid.*, 1963, v15, 202.
5. ———, *ibid.*, 1962, v14, 230.
6. ———, *ibid.*, 1962, v14, 471.
7. ———, personal communication.
8. Glimstedt, G., *Acta Pathol. Microbiol. Scand.*, 1936, suppl. XXX.
9. Gustafsson, B. E., *ibid.*, 1948, suppl. LXXIII.
10. Miyakawa, M., *Le Sang*, 1957, v28, 698.
11. Thorbecke, G. J., *Ann. N. Y. Acad. Sci.*, 1959, v78, 237.
12. Gordon, H. A., *Am. J. Digest. Dis.*, 1960, v5, 841.
13. Bauer, H., Horowitz, R. E., Levenson, S. M., Popper, H., *Am. J. Path.*, 1963, v42, 471.
14. Gustafsson, B., *Ann. N. Y. Acad. Sci.*, 1959, v78, 17.
15. Pool, J. G., Robinson, J., *Brit. J. Haemat.*, 1959, v5, 17.
16. Jacobsson, K., *Scand. J. Clin. Lab. Invest.*, 1955, suppl. XIV.
17. Godal, H. C., *ibid.*, 1961, v13, 530.
18. Hjort, P., Rapaport, S. I., Owren, P. A., *J. Lab. Clin. Med.*, 1955, v46, 89.
19. Waaler, B. A., *Scand. J. Clin. Lab. Invest.*, 1959, suppl. XXXVII.
20. Stormorken, H., *ibid.*, 1957, v9, 273.
21. Stapp, W. F., *ibid.*, 1958, v10, 169.
22. Owren, P. A., 1963, personal communication.
23. Hjort, P. F., *Scand. J. Clin. Lab. Invest.*, 1957, suppl. XXVII.

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Mouse Tissue Isoantigen Detectable by Immunoprecipitation.* (29605)

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Isoantigenic variants of mouse gamma globulin (allotypes) as well as other mouse serum isoantigens have been demonstrated by immunoprecipitation(1-3). *Mouse heteroantigens* of cellular origin have also been shown to react with appropriate antibodies from a foreign species in gel-diffusion tests(4,5). Mouse cellular *isoantigens,* however, have to our knowledge been detected only by other techniques, such as hemagglutination, hemagglutination-inhibition, leukocyte agglutination, immuno-fluorescence, cytotoxicity, induction of tumor homograft enhancement or accelerated homograft rejection. We now report that certain mouse sera obtained after viral oncolysis of a transplanted Ehrlich ascites tumor contain an antibody which precipitates with aqueous extracts from certain mouse tumors and similar extracts from normal organs of several mouse strains.

Materials and methods. Inbred A2G mice and random-bred ICR mice were purchased from Dublin Laboratories, Dublin, Va. Other inbred mice and F_1 hybrids were purchased from Jackson Laboratories, Bar Harbor, Maine. Coisogenic B10.A and C3H.K mice were obtained from Dr. G. D. Snell, Jackson Laboratories.

Ehrlich ascites tumor and the conditions for oncolysis by WSA influenza virus were as previously described(6). The Krebs 2

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