ducts were bare. Two of these 3 glands were from rats that had lost weight during the experiment. This latter condition of the mammary glands is similar to that observed by Reece and Leonard[6] following either estrogen injection into partially hypophysectomized (minute fragment) rats or estrogen and growth hormone injections into hypophysectomized rats. The results are summarized in Table I.

These results indicate again that estrogens will not stimulate duct growth of the mammary glands of hypophysectomized rats. The main action of lactogen, when injected alone, appears to be that of causing a thickening of the duct system along with a tendency to prevent the regression of the lateral buds. In a single case, lactogen injection caused end buds to proliferate. Our observations suggest that the lactogenic hormone is the pituitary factor which permits mammary duct growth in the rat. These observations agree with other known facts, since it has been shown that the lactogen content of the pituitary gland increases considerably at sexual maturity,[7] a time when the mammary duct system is rapidly proliferating. Moreover, estrogen injections into ovariectomized rats augment the lactogen content of the pituitary gland,[8] making possible the development of the duct system.

Summary. In castrated-hypophysectomized rats estrogen did not stimulate mammary duct growth. The injection of lactogenic hormone caused a thickening of the duct system and in combination with estrogen it induced mammary duct growth similar to that seen in intact rats following estrogen injections.

[6] Reece, R. P., and Leonard, S. L., *Endocrinology*, 1941, **29**, 297.

[7] Reece, R. P., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1937, 266.

[8] Reece, R. P., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 283.

15002

Serologic Agglutination of Antigen-Coated Dye Particles.

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Previous reports[1,2] have indicated the possible utility of a serologic reaction wherein the antigen as *e.g.*, horse serum or neurotropic virus, has been adsorbed to heat killed bacterial cells (*Serratia marcescens*) and in this form, employed as an agglutinable suspension. The technic of the test (B.A. or bacterial agglutination method) in its present state of development[3] is one, which for satisfactory operation, particularly with viral antigens, requires that scrupulous attention be given to various details in its performance. Some difficulty may be experienced also in observing a reaction, even under optimal conditions, since the masses of agglutinated bacteria are very small and may not be easily recognized. It was considered that certain of the critical limitations or deficiencies of the B.A. method could be obviated by substituting for *S. marcescens* cells, other types of particulate matter possessing similar adsorptive capacities but of units larger in dimension. Also it was expected that a particulate substance might be found which would serve the purpose without involving some of the disadvantages encountered in the use of collodion pellets[1] as for instance, the necessity for careful removal of electrolyte from antigenic material, by dialysis, as a preliminary to its adsorption. Preliminary experiments indicated that par-

[1] Roberts, E. C., and Jones, L. R., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 11.

[2] Roberts, E. C., and Jones, L. R., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 52.

[3] Roberts, E. C., *J. Immunol.*, 1945, **50**, 55.

ticles of carmine[4] possessed this advantage when used with horse serum as the antigen and in the experiments covered in the present report suspensions of this dye have been used as the particulate material.

In coating the particles of carmine with horse serum the routine technic has been developed after a great many preliminary experiments dealing with various factors which are involved in satisfactory performance of this type of test. Among these factors are that of particle size, ratio of particles to added antigen, dilution, time, temperature, pH and electrolyte concentration.[3] The technic, as it was finally developed, may be described as follows: 2 g of the dye suspended in 10 ml of water are ground in a mortar for 4 hours, whereupon, the suspension is centrifuged for one minute in a 52° angle centrifuge at 1750 R.P.M. (R.C.F. approximately 350). The sediment, which consists of the coarser particles, is discarded and the supernate is re-centrifuged for 6 minutes at 2750 R.P.M. (R.C.F. approximately 900). The sediment which is now collected contains particles of dye of about the proper size for satisfactory behavior in subsequent use. (Larger particles of the dye have been found to be less suitable because of greater sensitivity to the agglomerating effect of electrolyte.) A 1% suspension (by volume) is made in 0.25% sodium chloride solution which constitutes the stock suspension and has been found to keep well even upon prolonged storage. To "coat" these particles with the antigen, 4 ml of a 1:20 dilution of normal horse serum in 0.85% salt solution are added to 1 ml of the suspension; the mixture is gently shaken for 15 minutes at room temperature whereupon it is centrifuged at a rapid rate of speed, to insure complete sedimentation of the sensitized particles. The supernate is discarded and the sediment resuspended evenly in 5 ml of 0.25% salt solution. The antigen suspension is now ready for use and when stored at 5°C retains its usefulness for long periods of time. Each lot of suspension may be tested for specific agglutinability with serial dilutions of a standard antihorse serum. In our experience with various lots prepared at different times, the uniformity, as determined in this way, has been striking.

In making agglutination tests with antigen suspensions as prepared above and rabbit antihorse serum, we have found it convenient and satisfactory to employ thick glass slide with multiple depressions.* One drop of antigen suspension is placed in each depression and one drop of the antihorse serum (or a desired dilution thereof in 0.85% salt solution) is added, whereupon the slide is placed on the stage of a mechanical shaker, operating at a speed of 145 oscillations per minute, for 15 minutes and at room temperature. Suitable controls are included in each series of tests. Agglutination of the sensitized particles under these conditions is sharp, well defined and easily recognized. While with particles of this size the tests may be made in tubes, longer incubation periods in the water bath with higher temperatures are required and the end-titer of an antiserum is not increased. In the test tube method several hours of incubation at 28°C, or 43°C, are required for maximal agglutination which on the other hand occurs in 15 minutes when the open slide method is used in conjunction with the mechanical shaker.

When the sera of rabbits, previously injected with a single immunizing dose of horse serum are examined for the first appearance of specific antibody, employing agglutination of antigen-coated-carmine-particles (AACCP) wherein serial dilutions of the antiserum are used, and for comparison the conventional precipitation test employing serial dilutions of antigen, it has been found, as is indicated in Table I, that antibody can be detected from 2 to 6 days earlier by the former method. This early detection of antibody appears to be a particular merit of the AACCP method. However, as antibody production advances in a given animal the titer of the antiserum as expressed in terms of antigen dilution, is usually higher by the conventional precipitation method.

The validity of the AACCP method for

[4] Smorodintzeff, A. A., and Fradkina, R. V., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 93.

* The type commonly used in making the Kline test for the diagnosis of syphilis.

TABLE I.
Antibody Response in Rabbits Injected with One Dose of Horse Serum.

Rabbit No. (0.5 ml/kg intravenously)	Method	Pre-injection	Post-injection 2 da.	4 da.	6 da.	8 da.	12 da.
33	AACCP*	—	—	20	20	20	40
	Pp†	—	—	—	2	40	10240
34	AACCP	—	20	80	80	640	80
	Pp	—	—	—	2	5120	20480
43	AACCP	—	—	2	80	40	
	Pp	—	—	—	5120	20480	
44	AACCP	—	40	80	80	320	
	Pp	—	—	—	—	1280	
45	AACCP	—	—	2	2	40	
	Pp	—	—	—	—	1280	
46	AACCP	—	20	2	20	40	
	Pp	—	—	—	—	2560	
(10 ml/kg subscapularly)							
35	AACCP	—	20	40	80	160	1280
	Pp	—	—	320	1280	10240	20480
36	AACCP	—	20	40	80	160	1280
	Pp	—	—	320	640	5120	10240

* Agglutination of antigen (horse serum) coated carmine particles. Figures refer to the highest dilution of antiserum at which reaction occurred.

† Conventional precipitation reaction. Figures refer to highest dilution of antigen yielding a visible precipitate.

TABLE II.
Effect of Horse Serum (Antigen) on Carmine Particles Sensitized with Various Rabbit Anti-horse Sera.

Antiserum	AACCP titer of antiserum	Dilutions of horse serum (antigen) 1:2	1:5	1:10	1:20	1:40	1:80	1:160
No. 1	1:5120	xx	xx	xx	xx	x	x	—
No. 2	1:1280	xx	xx	xx	x	x	—	—
No. 3	1:20	—	—	—	—	—	—	—

the detection of humoral antibody would seem to be established since the agglutinating factor is not encountered in the pre-immunization sera of rabbits nor in the sera of rabbits immunized against non-related antigens. Further, in adsorption experiments wherein antigen-coated particles were added to homologous antisera it was found that all of the agglutinating factor (antibody) could be specifically removed.

In a limited number of experiments devoted to a study of the behavior of dye particles when used for this purpose it was found that antigen-sensitized particles are much less sensitive to the agglutinating effect of sodium chloride than are non-sensitized ones and accordingly the threshold concentration of electrolyte required for agglutination of the former appears to be related, quantitatively or otherwise, to the extent or character of the adsorption of horse serum antigen. If a particular suspension of antigen-coated particles agglutinated upon the addition of an equal volume of 3.4% sodium chloride (*i.e.*, 4×0.85) it was arbitrarily considered to be unsatisfactorily coated, to be poised too close to the electrolyte threshold at which non-specific agglutination would occur, and accordingly was not employed in making tests. Other preliminary experiments revealed that carmine dye particles are susceptible to sensitization with both the albumin and globulin fractions of horse serum since particles sensitized with either of these fractions were agglutinated by antihorse serum. Also, it was observed that the process could be reversed, *i.e.*, particles could be satisfactorily coated with rabbit antihorse serum (antibody) and later agglutinated by the mere addition of horse serum (antigen.) The results of

typical titrations are given in Table II. It will be noted also that the ability of a given antiserum to sensitize carmine particles under these conditions is related to the antibody content of the serum employed.

With respect to the adsorption of antibody, the behavior of carmine particles differs possibly from that of collodion particles since Jones[5] has reported experiments wherein the latter were exposed to an immune serum, subsequently washed with alkaline salt solution, and later failed to agglutinate upon the addition of antigen. The difference in the ability of collodion particles to become sensitized with antibody as compared with that of carmine particles may be due to differences in the composition of these substances. On the other hand, in the experiments with collodion particles, antisera of less than adequate potency may have been used, or the triple washing with alkaline salt solution may have resulted in the elution of a significant amount of antibody.

In our experiments all rabbit blood specimens were allowed to clot, placed in the refrigerator without delay and the sera were removed and tested without heat inactivation. The tests were made usually on the same day, but in no instance more than 40 hours later. Accordingly, all specimens might be regarded as having been fresh ones. During the study of the AACCP reaction it became evident that there are certain differences in the behavior of fresh and heat inactivated sera. Experiments with this aspect of the study may be summarized as follows: the earliest antibody factor which appears after administration of antigen (often occurring in the 48 or 96-hour post-injection sample) and which is detectable only by the AACCP method, appears to be relatively heat labile (withstands 52°C, 30 minutes; destroyed by 56°C, 30 minutes) and to be susceptible to deterioration upon standing (4 days at 5°C). Later increments of the antibody response in a given animal, as observed in subsequent serum samples, are characterized by usual degree of stability to heat and aging, however, a labile antibody-factor is also present in these specimens. In our experiments it has not been possible to re-activate such heated or stored sera which originally possessed the labile antibody-factor by the inclusion of fresh normal serum in AACCP tests. On the other hand, as might be expected, the antibody-factor (precipitin) involved in the conventional precipitation reaction, appearing somewhat later in these immunized rabbits, was not affected by heat or storage. Further study is required to elucidate the full significance of the above observations.

The sensitivity of the AACCP method is probably attested to by the observation that it will detect antibody earlier in the course of the immunization procedure than will the conventional precipitation test employing the antigen dilution method. Further, the former method will permit the detection of antibody in a relatively high dilution of antiserum as compared to the inability of such antisera to cause visible precipitation when diluted beyond reasonably low levels. For example, precipitating antisera which fail to elicit a visible reaction in the precipitation test when diluted above 1:10 or 1:20 may manifest the AACCP reaction when diluted 1:1000 or higher. This particular aspect of the AACCP reaction is in accordance with the concept of the mechanics of flocculation as afforded by Zinsser[6] wherein it is noted that larger particles present much less surface area per unit of mass than smaller ones. Since antigen-antibody reactions are primarily surface phenomena it follows within the concept, that an agglutinating serum can be diluted many thousand times as highly before losing the ability to yield a visible agglutination, as it can be before losing the ability to yield a visible precipitate with an antigen dispersed in the form of very small units.

The experimental use of carmine particles as the possible nuclei of fabricated, non-soluble units of antigen would seem to be worthy of trial in the fields of viral infections, allergic disorders and other diseases wherein it is now difficult or impossible to demonstrate antibody mechanisms by conventional methods. In this connection, Smorodintzeff and Frad-

[5] Jones, F. S., *J. Exp. Med.*, 1928, **48**, 183.

[6] Zinsser, H., *J. Immunol.*, 1930, **18**, 483.

kina[1] have recently reported their success in using dye particles (carmine) for the adsorption of an antigen and of an antibody concerned in typhus fever.

Summary. By adsorption of equine serum (antigen) to suitable particles of carmine a suspension can be made which is specifically agglutinable in rabbit antihorse serum. With suitably prepared suspensions, the open slide method may be conveniently employed in making agglutination tests. Employing this technic it is possible to detect antibody in the fresh sera of immunized rabbits 2 to 6 days earlier than it is by the conventional precipitation test employing the antigen dilution method.

15003 P

Growth-Promoting and Growth-Inhibiting Factors in Rat Tumor Tissue.*

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The evidence as it exists today supports the view that cell growth-promoting properties of various tissue extracts cannot be correlated with the state of proliferation of the tissues from which they are made.[1] The generally accepted opinion that extracts of actively growing tumors display growth-promoting power to an exceptionally high degree, has also been shown to be erroneous. Tumor extracts appear to affect the rate of cell multiplication *in vitro* to the same extent as extracts of the respective normal tissues. In those species in which normal tissue extracts stimulated growth, tumor extracts acted likewise (*i.e.*, chicken, dog); in the species in which extracts of normal tissues did not affect cell proliferation the tumor extracts are also without growth-promoting power.[2]

The rat is a striking example of a species whose tissues are devoid of cell growth-promoting properties. Apart from extracts of brain, all remaining rat organs and tissues tested are ineffective in activating cell growth *in vitro*.[3] Rat tumors also display no growth-activating effect and even inhibit the proliferation of cell colonies.[2] The lack of growth-promoting power in extracts of normal and neoplastic rat tissues can be explained by either of the following assumptions: (a) Growth-promoting principles are absent in tissues of rats. (b) They are present but their activity is counteracted by coexisting inhibitors. The experiments reported below show that the absence of growth-promoting ability in extracts of rat tumors can be explained in accordance with the second hypothesis.

Experimental. In this study the procedure previously reported for the partial purification of the active principle from growth-promoting tissue extracts was used.[4] The experiments were performed with a benzopyrene sarcoma of rats, extracts of which have a definitely inhibiting effect on the growth of fibroblast colonies *in vitro*.[2] Tumor tissue was finely minced in the Latapie apparatus and the pulp obtained was extracted with 4 volumes of Ringer's solution. The tissue extract was then

* Aided by a grant from the Dazian Foundation for Medical Research, New York.

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1 Towell, O. A., and Willmer, E. N., *J. Exp. Biol.*, 1939, **16**, 60; Doljanski, L., and Hoffman, R., *C. R. Soc. Biol.*, 1939, **130**, 1246; Hoffman, R., Tenenbaum, E., and Doljanski, L., *Nature*, 1939, **143**, 764; Hoffman, R., and Doljanski, L., *Growth*, 1939, **3**, 61; Hoffman, R., Tenenbaum, E., and Doljanski, L., *Growth*, 1940, **4**, 207.

2 Doljanski, L., Hoffman, R., and Tenenbaum, E., *Growth*, 1944, **8**, 13.

3 Hoffman, R., *Growth*, 1940, **4**, 361.

4 Werner, H., and Doljanski, L., *Nature*, 1942, **150**, 660.