differs from that seen after addition of cortisol + mammotropin to the culture medium (1,2). Cortisol + mammotropin-treated alveoli contain many intracellular vacuoles and a dense, darkly-stained secretion in alveolar lumina (Fig. 6), in contrast to loss of intracellular vacuoles and the unstained nature of luminal contents following cortisol + insulin treatment. Thus, there is more than one hormonal combination that will maintain prelactating and nodular alveoli in culture, but there are differences in the resulting histologic picture.

Summary. Cortisol plus insulin, and to some extent insulin alone, maintained normal and hyperplastic mouse mammary lobules in organ culture in a defined medium.

I am grateful to Drs. H. A. Bern and K. B. DeOme for suggestions, and to Victor Duran for photography.

1. Elias, J. J., *Science,* 1957, v126, 842.
2. Elias, J. J., Rivera, E., *Cancer Research,* in press, 1959.
3. Bern, H. A., DeOme, K. B., Alfert, M., Pitelka, D. R., *Second Internat. Symp. on Mammary Cancer,* Perugia, 1958, 565.
4. Chen, J. M., *Exp. Cell. Res.,* 1954, v7, 518.
5. Morgan, J. F., Morton, H. J., Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.
6. Harkness, M. N., Bern, H. A., Alfert, M., Goldstein, N. O., *J. Nat. Cancer Inst.,* 1957, v19, 1023.
7. Trowell, O. A., *Exp. Cell Res.,* 1959, v16, 118.

Received May 18, 1959. P.S.E.B.M., 1959, v101.

Prevention by Intravenous Injection of Antigen and Antibody of Passive Arthus Reaction to Unrelated Immune System. (24996)

OTTO G. BIER AND MARIA SIQUEIRA (Introduced by Z. Ovary)
Depts. of Microbiology, Escola Paulista de Medicina and of Immunology, Inst. Biológico, São Paulo, Brazil

Previous work has shown that intravenous injection of antigen followed by its specific antibody resulted in marked decrease of passive cutaneous anaphylactic (PCA) response to an unrelated immune system in the rat(1). The possible dependence of this suppressive effect upon adequate levels of circulating complement (C′) has been suggested(2), since intensity of PCA paralleled *in vivo* fixation of C′ under a variety of experimental conditions. The present report concerns observations along similar lines in relation to direct Arthus reactions passively induced in guinea pigs.

Materials and methods. Antigens and antisera. The following antigen preparations were employed: 1. Bovine serum albumin (BSA), crystallized, obtained from Pentex, Kankakee, Ill., used without further purification. 2. *Chicken egg albumin* (Ea), recrystallized 5 times, prepared by method of Kekwick and Cannan(3). 3. *Rabbit anti-BS,* lots 23, 24, 27 and Pool I, '58, containing 0.479, 1.080, 0.263, and 0.362 AbN/per ml respectively. 4. *Rabbit anti-Ea,* lots 11, 15, and 17, containing 0.746, 1.412, and 1.197 mg AbN/per ml respectively. Antibody content of antisera was determined by quantitative precipitin method described in reference 4. *Complement titrations.* Serum samples for C′ titrations were obtained from blood (1-2 ml) drawn by cardiac puncture. Sera were clarified by centrifugation at 0°C and stored at −20°C until assayed. Estimations of hemolytic activity were carried out as described (5). *Hematologic determinations.** 2 ml samples of blood were obtained by cardiac puncture with silicone-coated syringe and needle and collected into siliconed vial containing 0.025 ml of 10% solution of sodium versenate (EDTA). Leucocyte counts were performed as usual. For platelet counts the method of Feissly and Ludin, modified by Rosenfeld(6) was adopted. Blood was diluted 1:200 in siliconed red cell pipette using

* The authors are indebted to Drs. M. Lindenberg and P. Azevedo for carrying out hematologic determinations. The help of Dr. G. Rosenfeld is also gratefully acknowledged.

as diluting fluid a solution containing 0.05% sodium EDTA, 0.2% NaCl, and 3% cocaine hydrochloride. After careful mixing of contents, pipettes were left ½ hour at room temperature, then mechanically shaken for 3 minutes. A counting chamber of Levy-Neubauer type was filled with hemolysed content of pipette after discarding first drops and the homogeneously distributed, non-agglutinated platelets were counted under phase microscope over area of 2 mm². *Passive Arthus reactions* were produced by injecting 0.100 - 0.400 mg AbN (in 1 ml) by intravenous route followed immediately thereafter or 30 minutes later by 0.025-0.100 mg antigen N (in 0.1 ml) into skin of abdominal area of guinea pigs of 240-260 g. Animals were killed 2 hours after intradermal injection of antigen and reactions were read on undersurface after reflecting abdominal skin. Intensity of reaction was graded as recommended in(7), according to diameter of confluent or petechial hemorrhagic area: ++++ (more than 20 mm), +++ (15-20 mm), ++ (10-15 mm), + (5-10 mm), ± (less than 5 mm), and 0 (no reaction). The following experimental model was used in experiments such as in Table I:

0 hr	Intrav. inj. of "desensitizing" immune-system.
½	Bleeding for estimating C′ titer.
¾	Intrav. inj. of "sensitizing" (Arthus-producing) antiserum.
1¼	Intradermal inj. of Arthus-producing antigen.
3¼	Reading of results.

Intrav. inj. were performed with short-beveled gauge 26 needles into superficial veins of hind foot or of ear.

Results. A. *Influence on Arthus responsiveness of C′-depletion by previous intravenous injection of antigen and antibody.* Table I summarizes data on magnitude of passive Arthus reaction to intravenously injected anti-Ea (0.400 mg AbN) and intradermally injected Ea (0.025 and 0.050 mg EaN) in 3 groups of guinea pigs intravenously inoculated 45 minutes before with 0.200 mg BSA N plus 0.400 mg anti-BSA N or with either one of preceding reagents. A fourth group of non-injected controls was also included. Reactions of maximal (++++) or sub-maximal severity (+++) were observed in all animals, except those injected with BSA plus anti-BSA in which a clear cut suppressive effect was obtained. Complement titers in last group corresponded to approximately 10% of units present in other groups.

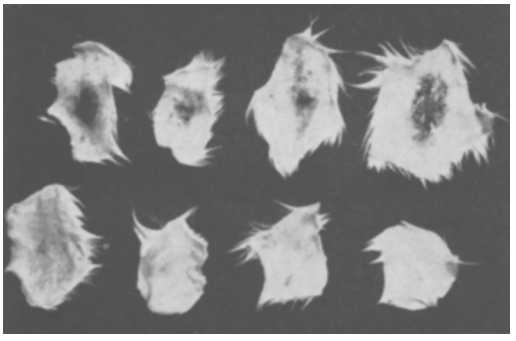

FIG. 1. Prevention by intrav. inj. BSA-anti BSA of Arthus reaction to intrav. inj. anti-Ea (.4 mg AbN) and intraderm. inj. Ea (.03 mg N). Skins in upper row are from non-desensitized guinea pig controls.

Identical results were obtained by using BSA-anti-BSA as C′-depleting system and Ea-anti-Ea as the Arthus producing system.

To investigate possible correlation between suppressive effect and complement drop effected by intravenous injection of antigen and antibody, an experiment was performed in which 3 different amounts of Ea/anti-Ea were intravenously injected one hour before sensitizing dose of anti-BSA. Pertinent data are

TABLE I. Prevention by Intravenously Injected BSA and Rabbit Anti-BSA of Direct Arthus Reactions in Guinea Pigs Sensitized with Rabbit Anti-Ea.

Amt of BSA and/or anti-BSA inj. intrav., mg N		Intensity of Arthus reaction 2 hr after intrad. inj. of Ea .050 mg N	Ea .025 mg N	C′ titers
I.	.20 BSA followed immediately by .40 anti-BSA	0	0	17
		±	±	26
		0	0	25
		±	±	25
II.	.20 BSA	4+	4+	267
		4+	4+	310
III.	.40 anti-BSA	4+	3+	214
		3+	3+	117
IV.	None	4+	4+	247
		4+	4+	227

Animals sensitized with .40 mg anti-Ea N 45′ after intrav. inj. of BSA/anti-BSA. Bleedings performed 15′ before sensitization and intradermal inj. of Ea 30′ thereafter.

TABLE II. Amount of Intravenously Injected Ea and Anti-Ea Required to Prevent Passive Direct Arthus Reactions to BSA/Anti-BSA

Amt of intrav. inj. Ea and anti-Ea, mg N	Intensity of Arthus reaction 2 hr after intrad. inj. of BSA .06 mg N	BSA .03 mg N	C′ titers
.20 Ea	0	0	20
.40 anti-Ea	0	0	16
	0	0	28
.10 Ea	0	0	48
.20 anti-Ea	±	0	40
	0	0	29
.05 Ea	±	±	118
.10 anti-Ea	+	+	85
	+	±	69
.40 anti-Ea	4+	3+	221
	2+	2+	207
	4+	3+	219

Animals sensitized with .40 mg anti-BSA N 1 hr after intrav. inj. of Ea/anti-Ea. Bleedings performed 20′ before sensitization and intrad. inj. of BSA immediately thereafter.

given in Table II. A very marked suppressive effect was obtained even in animals injected with 0.05 mg EaN plus 0.1 mg anti-EaN. C′ titers were normal in control group which received only 0.4 mg of anti-Ea N, whereas in other groups the reduction of C′ titer paralleled the amount of intravenously injected Ea/anti-Ea, *i.e.*, about 50% of normal titer in animals which received least amount of C′-depleting immune system.

Another way of investigating possible dependence of C′ levels upon suppressive effect described here, was to study the influence of previous Arthus reaction in animals sensitized by intravenous injections of 2 different antibodies. Table III reproduces results obtained in 2 groups of guinea pigs which received a mixture of 0.4 mg anti-Ea N + 0.4 mg anti-BSA N by intravenous route and tested intradermally 30 and 90 minutes later with each of corresponding antigens (0.100 mg N) in alternate order.

Table III shows that the first reaction was always of greater severity than the second. However, although a complement reduction to approximately 20-40% of normal titer was observed as a consequence of first antigen-antibody reaction, only a slight reduction in severity of second reaction was noted, particularly in the group in which Ea was injected before BSA. These results are contrasted with those given in Table II in which a very marked suppressive effect was detected even in presence of 69 to 118 units of C′.

The possibility of restoring Arthus responsiveness in C′ depleted animals by injecting fresh guinea pig serum was also investigated. Two groups of guinea pigs were decomplemented by intravenous injection of Ea/anti-Ea. Animals of one of the groups were "recomplemented" with 5 ml of fresh guinea pig serum and passive Arthus reactions were provoked in both groups by intravenous injection of 0.4 mg anti-BSA N followed by intradermal injection of 0.06 mg BSA N, 90 minutes after decomplementation.

Results showed that the suppressive effect could not be overcome by recomplementation, although C′ titers rose from 49-61 $C'H_{50}$ in the decomplemented group to 124-142 units in recomplemented animals.

B. *Duration of suppressive effect and its relationship to number of circulating leucocytes and platelets.* Since *in vivo* C′ fixation is accompanied by marked leucopenia(8), it was of interest to study variation in number of circulating leucocytes and platelets in relation to time of duration of the suppressive effect. Repeated experiments showed that the suppressive effect produced by intravenous injection of antigen and antibody is still very marked after 3 hours. After 6 hours, reactions were usually of same severity in decomplemented and control groups.

Experiments were performed to follow the

TABLE III. Severity of Arthus Reactions in Guinea Pigs Injected Simultaneously with Anti-Ea and Anti-BSA by Intravenous Route and Tested Intradermally 30 and 90 Minutes Later with Each of Corresponding Antigens in Alternate Order

Order of intrad. inj.	Reactions 3 hr after 1st intrad. inj. .10 mg EaN	.10 mg BSA N	C′ titers
Ea—BSA	2+	±	42
	4+	2+	77
	4+	3+	
	4+	3+	
BSA—Ea	±	4+	53
	2+	4+	113
	2+	4+	82
	2+	4+	

Intrav. inj. of mixture of .40 mg anti-Ea N plus .40 mg anti-BSA N. Bleedings 10′ before second intrad. inj.

TABLE IV. Effect of Intravenously Injected Antigen and Antibody on Circulating Leucocytes and Platelets.*

Group	Time between inj. of BSA/anti-BSA and bleeding for leucocyte and platelet counts (hr)	Total leucocytes/mm³	Platelets/mm³ × 1000
A. Inj. intrav. with .2 mg anti-BSA N followed by .4 mg BSA N	None	980	0
		680	0
	2	9720	148
		6300	94
	4	6500	164
		8580	146
	6	6400	142
		8740	301
B. Non-injected controls	—	9720	346
		5510	324

* Results above refer to individual animals.

number of circulating leucocytes and platelets at 0, 2, 4, and 6 hours after decomplementation. The number of circulating leucocytes falls to approximately 10% immediately after intravenous injection of antigen and antibody but becomes normal again after 2 hours (Table IV). The effect on platelets is more striking and recovery slower, approaching normal values after 6 hours.

Discussion. The fact that no correlation was found between lack of Arthus response and number of C′ units in circulating blood when the Arthus-producing system was injected (Cf Tables II and III), seems to indicate that C′ depletion is not a causal factor in abolishment of the reaction.

Stone(9), pointing out that guinea pigs are difficult to decomplement by injection of antigen-antibody combinations, also reached the conclusion that C′ was not the critical factor in his experiments on inhibition of Arthus reaction during protracted anaphylactic shock.

Since "desensitizing" effect of C′-depleting immune system may depend on a critical concentration of certain C′-component(s) not expressed by whole hemolytic titers as in present experiments. Corroborating evidence against the rôle of C′ is given by failure of recomplementation in restoring Arthus reactivity in C′-depleted animals.

The possible rôle of platelets is more strongly suggested. Immune adherence of platelets on specific precipitate used for "desensitization" would result in a transitory deficiency of these elements at site of intradermal injection of antigen and thus prevent local vascular effects essential for development of the hemorrhagic reaction. This would be in logical agreement with Stetson's interpretation(10) of the mechanism of Arthus and Shwartzman reactions.

It is apparent, however, that a final explanation of our findings awaits further investigations.

Summary. 1. Previous intravenous injection of antigen and antibody prevents development of passive Arthus reaction to an unrelated immune system in guinea pigs. 2. This suppressive effect does not seem to depend on C′ depletion but rather on deficiency of platelets in circulating blood.

1. Bier, O. G., Siqueira, M., Osler, A. G., *Int. Arch. Allergy and Appl. Immunology,* 1955, v7, 1.

2. Osler, A. G., Hawrisiak, M. M., Ovary, Z., Siqueira, M., Bier, O. G., *J. Exp. Med.,* 1957, v106, 811.

3. Kekwick, R. A., Cannan, R. K., *Biochem. J.,* 1936, v30, 227.

4. Kabat, E. A., Mayer, M. M., *Experimental Immunochemistry,* Springfield, Ill., C. C. Thomas, 1948.

5. Osler, A. G., Strauss, J. H., Mayer, M. M., *Am. J. Syph. Gon. Ven. Dis.,* 1952, v36, 140.

6. Spanoudis, S., Eichbaum, F., Rosenfeld, G., *J. Immunol.,* 1955, v75, 167.

7. Benacerraf, B., Kabat, E., *ibid.,* 1950, v64, 1.

8. Stavitsky, A. B., Stavitsky, R., Ecker, E. E., *ibid.,* 1949, v63, 389.

9. Stone, S. H., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 269.

10. Stetson, C. A., *J. Exp. Med.,* 1951, v94, 347.

Received December 31, 1958. P.S.E.B.M., 1959, v101.