No interferon has been found in pools of M and S variants made in these cells.

Summary. Interferon production in mouse embryo fibroblast cultures infected with the highly oncogenic S variant and the poorly oncogenic M variant of polyoma virus has been studied. Infection of cultures by the M variant was associated with a 4-fold or greater production of interferon by the infected cells. Cultures infected with the S variant, however, produced higher titers of virus. These results may explain several findings such as the differing plaque size of polyoma virus variants of high and low oncogenicity, and suggest a possible relationship between the differing oncogenicity in mice of these virus strains and the variation in interferon production caused by infection of mouse embryo fibroblast cultures by them.

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Hyperreactivity to Endotoxin in BCG Treated Guinea Pigs and Its Relationship to Delayed Hypersensitivity.* (28041)

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Hyperreactivity to endotoxins of Gram negative bacteria has been studied by many investigators(1,2,3). Recently, Suter(4) described the increased reactivity to endotoxin following BCG immunization of mice. The present investigation was undertaken to clarify the relationship of delayed hypersensitivity to such hyperreactivity in BCG vaccinated guinea pigs, animals more amenable to the study of delayed hypersensitivity.

Materials and methods. Male guinea pigs of the Hartley strain, weighing between 450 and 550 g, maintained on a commercial chow diet† were used.

Immunization. Ten- or 11-day-old cultures of BCG in albumin growth medium were injected intraperitoneally to produce hyperreactivity and sensitivity. Human gamma globulin‡ (HGG) was denatured by heating at 60°C for 60 minutes. For immunization, equal parts of denatured human gamma globulin (DHGG) in saline and incomplete adjuvant§ were mixed. 0.1 ml of the mixture was injected into each of the 4 footpads of the guinea pigs. At times 0.4 ml was injected intraperitoneally. Total dose was 20 μg per guinea pig. With such a course of immunization, good delayed type skin reactivity to DHGG has been observed as early as 5 days after the initial injections.

Test substances. Escherichia coli 0111:

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† Purina Chow, Ralston Purina Co., St. Louis.

‡ Pentex, Inc., Kankakee, Ill.

§ Difco Laboratories, Inc., Detroit, Mich.

TABLE I. Systemic Reactivity to Endotoxin and PPD in BCG* Immunized Guinea Pigs.

	Endotoxin			PPD		
Day tested†	Total dose (μg)	μg/100 g	Results	Total dose (μg)	μg/100 g	Results
6	100	18 -22	4/4	2500	450-550	0/4
	50	9 -11	4/4			
	10	1.8- 2.2	2/3			
12	100	18 -22	4/4	2500	450-550	1/4
	50	9 -11	3/4	1250	225-275	0/4
	10	1.8- 2.2	1/4			
16	100	18 -22	3/4	2500	450-550	1/4
	50	9 -11	2/4	1250	225-275	0/4
	10	1.8- 2.2	0/4	500	90-110	0/3
21	200	36 -44	2/4	2500	450-550	2/4
	100	18 -22	1/3	1250	225-275	1/4
	50	9 -11	0/3	500	90-110	1/4
	10	1.8- 2.2	0/4			
26	300	54 -66	3/4	2500	450-550	1/3
	200	36 -44	2/4	1250	225-275	1/4
	100	18 -22	1/3	500	90-110	1/4
	50	9 -11	0/4			
32	300	54 -66	3/4	2500	450-550	1/3
	200	36 -44	2/3	2500‡		2/4
	100	18 -22	1/3	1250	225-275	2/4
				500	90-110	0/2
40	300	54 -66	2/5	2500	450-550	2/4
	200	36 -44	1/5	1250	225-275	2/4
				500	90-110	2/4

* .5 ml BCG intraperitoneally. † After immunization. ‡ Intraperitoneally.

B4 endotoxin§ dissolved in pyrogen-free saline and sterilized by heating in a boiling water bath for 10 minutes and Purified Protein Derivative (PPD)‖ dissolved in pyrogen-free saline and sterilized by filtration were used as test materials. To produce systemic reactions, both substances were administered *via* penial vein or intracardially.

In vitro *tests*. For *in vitro* sensitivity testing, spleen fragments in chicken plasma clots in Leighton tubes were overlaid with 1.0 ml of PPD or endotoxin diluted in a 10% chick embryo extract—10% normal guinea pig serum—80% Hanks' balanced salt solution medium. Tubes were read after suitable periods of incubation at 37° for density and extent of migration of large wandering cells. Tubes containing test substances were compared with a control series without test substances and ranks recorded. Statistical analyses were carried out according to the method of White(5) as applied to such *in vitro* testing(6,7,8).

‖ RT-23, State Serum Inst., Copenhagen.

Results. Studies of the systemic reactivity of normal animals demonstrated that the 50% lethal dose of endotoxin was between 65 and 90 μg per 100 g body weight whereas 900-1100 μg of PPD per 100 g body weight were non-toxic. Many animals treated with endotoxin appeared quite ill but subsequently recovered. This was never observed with PPD treated normal animals. Results of an experiment utilizing BCG immunized guinea pigs are recorded in Table I. Increased reactivity to endotoxin was clearly demonstrable shortly after administration of BCG with maximum reactivity between 6-12 days. Reactivity was increased about 40- to 50-fold at 6-12 days, about 10-fold at 16 days, and finally was increased only approximately 2-3-fold at 21-26 days. Slight hyperreactivity was demonstrable at 30-40 days. During the period of hyperreactivity, deaths occurred within 2-3 hours after injection of endotoxin. Ordinarily, deaths were observed much later in animals with normal reactivity. Autopsy revealed areas of hemorrhage around the caseous lesions produced as a result of BCG in-

TABLE II. Systemic Reactivity to Endotoxin in Guinea Pigs Immunized with Denatured Human Gamma Globulin (DHGG).

Day tested (after immunization)	Treatment	Total dose	μg/100 g	Results
8	DHGG in adjuvant* into foot pads	300	55-65	1/4
		200	35-45	1/4
		100	18-22	0/4
8	Adjuvant only into foot pads	300	55-65	1/3
		200	35-45	0/4
		100	18-22	0/4
8	DHGG intraperitoneally	300	55-65	1/4
		200	35-45	0/3
		100	18-22	0/3
8	Adjuvant only intraperitoneally	300	55-65	1/3
		200	35-45	1/4
		100	18-22	0/3

* Incomplete adjuvant.

jection, particularly on the omentum, the site of most such lesions. Normal animals dying following endotoxin injection showed the characteristic lesions of stomach and gut wall. Systemic reactivity to PPD appeared after 12 days and increased to a maximum stationary level after about 21 days, while hyperreactivity to endotoxin declined noticeably. Table II records the systemic reactivity to endotoxin of guinea pigs immunized with DHGG. Such animals showed delayed type skin reactions to human gamma globulin or DHGG (9,10). No change in susceptibility to systemically administered endotoxin is apparent. *In vitro* sensitivity tests (Table III) revealed that cellular sensitivity to endotoxin at the time of hyperreactivity was not increased and indeed cellular reactivity to endotoxin seemed somewhat lower. Cellular reactivity to endotoxin seems to fluctuate somewhat but no suggestion that increased cellular reactivity can account for the systemic hyperreactivity was found. However, cellular sensitivity to PPD can be demonstrated only 14 days subsequent to immunization and the level of sensitivity increases to a maximum at 24 days, the time of maximum systemic reactivity. Thereafter, the level remains fairly constant until 38 days, when the experiment was terminated.

Discussion. The time pattern of development of hyperreactivity to endotoxin is entirely different from that of development of specific delayed hypersensitivity to PPD. Indeed, at the time of maximum hyperreactivity, neither cellular nor systemic reactivity to PPD can be demonstrated, although high levels of sensitivity to PPD can be demonstrated later when hyperreactivity to endotoxin is essentially normal. Hyperreactivity to endotoxin is manifested in these animals by an intense local hemorrhagic reaction surrounding caseous nodules containing BCG which had developed primarily on the omentum. Systemic reactivity to PPD can be demonstrated at about the same time that cellular sensitivity appears as shown by the *in vitro* tests; thereafter, both increased in parallel. During this time, the caseous lesions regressed and local hemorrhagic reactions around the nodules, following administration of endotoxin, became less and less prominent. Finally, even in the absence of lesions due to BCG, systemic reactivity to PPD is still demonstrable.

Injection of denatured human gamma globulin leads to production of delayed hypersensitivity to denatured and undenatured human gamma globulin as demonstrated by skin tests 5 days after injection. *In vitro* tests also indicate a development of cellular sensitivity. These experiments will be reported in detail elsewhere. Nevertheless, such animals tested at 8 days, a time at which hyperreactivity, if it existed, would be expected to be maximal, show no evidence of hyperreactivity to endotoxin.

The production of hyperreactivity to endotoxin by such diverse means as injection of thorotrast, trypan blue, and colloidal carbon (2,11), infection of the animal with group A streptococci(12), the injection of cord factor (4), and the fact that this hyperreactivity can be elicited by the use of endotoxins of various species of bacteria indicate that cellular and systemic reactivity is non-specific. Such cannot be the case for cellular and systemic reactivity to PPD. Nevertheless, investigators seem reluctant to separate the phenomena of hyperreactivity to endotoxin, endotoxin sensitivity, and delayed sensitivity. Our data clearly indicate that hyperreactivity and delayed sensitivity have different basic mechanisms. Hyperreactivity is probably due to

TABLE III. *In vitro* Sensitivity of Splenic Explant Cells of Individual Animals to PPD and Endotoxin.

Days after BCG immunization	PPD concentration†			Endotoxin concentration†		
	25	6.3	1.6	6.3	1.6	0.16
Non-immunized*	—			—		
	—			+	—	
	—			+++	++	—
	—			+++	++	—
	—			+++	++	—
6				—		
				—		
				+	—	
10				—		
				—		
				++	—	
12	—			—		
	—			—		
	—			+	—	
14	+	—		—		
	++	+	—	—		
	+++	++	—	—		
19	—					
	++	—				
	+++	++	+++	+++		
24	++	++	+	++	+	—
	+++	+++	++	—		
	+++	+++	+++	++		+++
28	+++	++	++	++	—	
	+++	+++	+	+++	+	++
38	+++	+++	+++	++	+++	+

— = $p > .05$
\+ = $p \leq .05 > .01$
++ = $p \leq .01 > .001$
+++ = $p \leq .001$

$p \leq .05$ indicates that test explants differed significantly from control explants.

* Tested 28-39 days after start of experiment.

† μg/ml in overlay medium.

a local reaction in an area of intense response to the bacterial infection.

Summary. The development of systemic and cellular reactivity to PPD has an entirely different pattern from that of hyperreactivity to endotoxin in BCG immunized guinea pigs. Hyperreactivity appears not to be related to altered cellular reactivity in contrast to PPD sensitivity or delayed sensitivity to well characterized proteins.

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