dose of 100 μg of DMBA, on the other hand, is a borderline carcinogen giving rise to a small proportion of papillomas many of which regress and few of which progress to malignancy. This result was confirmed. From other studies it is well known that a dose of 100 μg is an effective initiating dose of this carcinogen for the skin; that is, if it was followed by repeated applications of croton oil or some other promoting stimulus, many tumors would appear(10,11). In the present study this dose of carcinogen was combined with a single application of croton oil at 2 dosages. In no instance did croton oil, thus administered, give rise to any promoting effect, but to the contrary appeared, if anything, to engender a reduction in tumor incidence. This finding applied not only to combinations with the 100 μg dose of DMBA, but also to the 200 μg dose.

It thus seems clear that the difference in the carcinogenic capabilities of doses of 100 μg or 200 μg of DMBA cannot be explained on the basis of additional "injury" inflicted by the higher dose, if the "injury" is of the same type as that caused by croton oil. It is clear that the promoting effects of croton oil are quite distinct from the initiating and carcinogenic actions of DMBA since croton oil requires repeated applications to manifest its effect and has, if anything, the reverse effect under the conditions of the present expriment. The qualitative nature of the tumors seen in the croton oil treated groups appears consistent with that observed in many previous studies in that there was a predominance of benign and regressing lesions(2).

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Partial Protection of Mice by Human Gamma-Globulin Against *Staphylococcus aureus* on Subcutaneous Sutures.* (27090)

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Despite the world-wide challenge of staphylococcal infection, its mechanisms of pathogenesis are not well understood, and there is a dearth of measurable parameters for gauging specific resistance, particularly in man (1). Transfer of observations on staphylococcal infection from the experimental animal to man is subject to serious ambiguities(2). Elek and Conen(3) elaborated a test involving threading of infected sutures through the skin, which they applied to human subjects. James and MacLeod(4) have applied a slight modification of Elek's technic to experimental work in mice in the hope that this would provide a model system less artificial than some other procedures currently in use. In the present study we have examined some effects of pooled human gamma globulin in modifying response to infected sutures in mice, following the procedure of James and MacLeod. It would appear that this system of infected sutures is indeed a useful model.

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Materials and methods. The strain of *Staphylococcus aureus* employed was that of Kapral and Li(5), 18-ZA. This strain, phage type 42B/52/80/81, possesses soluble and bound coagulase, fibrinolysin, hyaluronidase, alpha and delta hemolysins, and leukocidin.

Preparation of infectious sutures. Silk sutures (size 4-0, code A-53, Ethicon) 2 inches in length, with ends previously dipped in collodion to prevent fraying, were sterilized by boiling. The sterile sutures were seeded with the organisms by immersing the suture for 30 min. at 37°C in a culture of *S. aureus*. The concentration of the culture was determined by a Klett reading. James and MacLeod(4) found that silk sutures would adsorb a number of bacteria approximating one thousandth the concentration of the original culture. After 30 minutes' incubation the sutures were removed and placed on a sterile filter paper in a petri dish and stored at 4°C until use the following day. The number of organisms per suture was estimated by shaking each of 10 sutures in trypticase soy broth on a general purpose shaker (Arthur H. Thomas Co.) for 45 min. at room temperature. Two-tenths ml of the broth from each tube was spread on a trypticase soy agar plate incubated at 37°C for 24 hours and the colonies counted. The ends, approximately 0.25 inch each, of 10 sutures were similarly treated and number of organisms per suture ends determined. The numerical difference between the two is taken as minimal average number of organisms per mouse.

Preparation and suturing of mice. White Swiss male mice (weight 14-16 g) were rendered unconscious by an 0.15 ml injection intraperitoneally of a 1/5 dilution of Sodium Nembutal (Abbott) during both depilation and suturing. The fur on the nape of the neck was removed by applying a depilatory (Nair) for a period of 3-5 min., then removing both fur and excess depilatory. The following day an infectious suture was drawn under the skin of the nape of the neck and the suture ends cut so that approximately ⅛ of an inch protruded from the skin. To prevent loss of the suture, a drop of collodion was placed over each suture end and the animals were housed in individual cages.

The mice were examined for purulent lesions each day for 5 days after suturing. An animal was considered positive if the suture could no longer be seen under the skin. On the fifth post-suture day each animal was autopsied and lesion, spleen, kidney, liver and heart blood were tested for presence of staphylococci.

Gamma globulin. The gamma globulin was prepared by the cold ethanol fractionation(6) of about 200 pooled plasmas. The final product was a 16% solution in 0.3M glycine, a concentration of about 20-fold of the gamma globulin of the original plasma pool. The gamma globulin had an agglutination titer to the parent (A) strain of 6,400 and an alpha-hemolysin titer of 15 units per ml.

Albumin. The albumin used was obtained from the Philadelphia Serum Exchange and prepared similarly to the gamma globulin. The human albumin was employed as a non-specific protein control in a small number of animals. The technic followed was exactly the same as that used for the gamma globulin experiment, except that albumin was used.

Results and discussion. Two hundred mice were sutured with infectious sutures. One hundred of the mice received 0.2 ml of gamma globulin at the following intervals: 18 hr and 2 hr prior to suturing, and 24, 48 and 72 hr post-suturing for a total of 1.0 cc of gamma globulin per mouse. Owing to the large number of mice required to establish a statistically valid experiment, small groups had to be employed; consequently, the number of organisms per mouse varied among the groups.

A 30% decrease in lesion formation was observed in the gamma globulin treated animals (Table I) when the infectious dose was small (*i.e.* 200-600 organisms per mouse). When animals received a high infecting dose (Table II) the group treated with gamma globulin developed approximately 35% fewer lesions. Table III and Fig. 1 depict the effect of gamma globulin when the data for all test animals are combined. In this consideration, the treated animals developed 31% fewer lesions than the control group.

Staphylococci could be recovered from the

TABLE I. Response of Treated and Control Animals Receiving Low Dose Infections.

No. of test animals	Avg No. of organisms per animal	Treated		Control	
		Pos.*	Neg.	Pos.	Neg.
46	200	9	16	14	7
17	282	6	4	6	1
37	400	6	11	13	7
30	600	3	7	14	6
† 130		24	38	47	21
% infectivity		38.7	61.3	69.1	30.9

* Animals were considered positive if a purulent lesion developed.
† Total

TABLE II. Response of Treated and Control Animals Receiving High Dose Infections.

No. of test animals	Avg No. of organisms per animal	Treated		Control	
		Pos.	Neg.	Pos.	Neg.
41	1000	14	14	12	1
29	1100	7	3	17	2
* 70		21	17	29	3
% infectivity		55.2	44.8	90.6	9.4

* Total

TABLE III. Response of Total Number of Treated and Control Animals.

No. of test animals	Avg No. of organisms per animal	Treated		Control	
		Pos.	Neg.	Pos.	Neg.
130	200- 600	24	38	47	21
70	1000-1100	21	17	29	3
* 200		45	55	76	24
% infectivity		45	55	76	24

* Total

positive animals, with a purulent lesion, only from site of inoculation. In the negative animals, devoid of purulent lesions, no organisms could be recovered from site of inoculation; however, if the suture was removed from the animal and cultured, the original organism could be recovered.

Statistical validity of experiment. Since the infectious dose varied over a range of 200-1100 cells per mouse (Table III) it was necessary to compare statistically those animals receiving a high infectious dose with those receiving a low infectious dose in both the treated and control groups (Table IV). In all instances except that of high *vs* low dose in the treated animals, the differences were significant.

Non-specific resistance. The mechanism by which gamma globulin enhances resistance to staphylococcal infection, that is by somatic or antitoxic antibodies, is not yet known in this system. However, it must be considered that non-specific factors may be involved. The fact that large amounts of foreign protein in the form of gamma globulin are injected into the treated animals might cause a non-specific type of protection, for instance by an increased infiltration of leukocytes resulting from the injected protein. No evidence for this hypothesis was elicited by injecting equal amounts of protein, in the form of albumin, in a small group of mice. The albumin-treated mice showed no increased resistance to staphylococcal infection, as assayed by this procedure, over those mice receiving no albumin. Work is presently being done to determine the specific nature of the factor or factors involved in the increased resistance reported here.

Discussion. A number of investigations using intraperitoneal challenge with staphylococci have demonstrated protection of mice by pooled human gamma globulin(7,8,9,10). Lambert(11) failed to demonstrate protection under the very artificial conditions of his experiments.

Beiser *et al.*(10) demonstrated multiple antistaphylococcal antibodies in pooled human gamma globulin, and Halbert *et al.*(12)

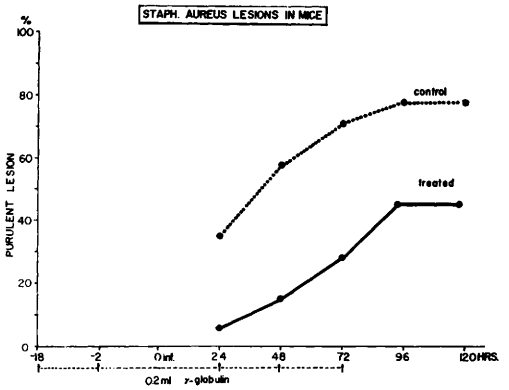

FIG. 1. Incidence of purulent lesions in mice with subcutaneously implanted sutures infected with *Staphylococcus aureus*, with and without administration of gamma-globulin.

TABLE IV. Statistical Analysis of Combined Experiments.

	High dose*	Low dose†	Treated	Control	
	Treated *vs* control	Treated *vs* control	High dose *vs* low dose	High dose *vs* low dose	Treated *vs* control
Chi square	8.98	10.90	4.30	7.50	18.60
Prob.	<.005	<.005	.50–.025	<.005	<.005

* High dose = 1000-1100 cells/animal. † Low dose = 200-600 cells/animal.

in the gamma globulin of cystic fibrosis patients. Potter and Stollerman(13) have demonstrated that adsorption of human gamma globulin (in particular Cohn Fraction II) to bentonite particles results in marked enhancement of phagocytosis of the particles by human leukocytes *in vitro*.

Experiments are in progress to identify the specific antibody or antibodies in gamma globulin protective against this cutaneous route of infection.

Summary. The technic of subcutaneous implantation of sutures infected with *Staphylococcus aureus* was used to assay protection of mice by pooled human gamma globulin. The mice which received 1 ml each of gamma globulin in divided doses showed 30 to 35% fewer purulent skin lesions than controls. The infected sutures, however, were not freed of viable staphylococci. This model system is regarded as being sufficiently satisfactory to warrant its continued use in experiments toward elucidation of critical factors in pathogenesis and protection.

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Stop Flow Analysis of Renal Tubular Site of Reabsorption of Radioiodide in Dogs.* (27091)

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The renal excretion of iodide in dog and man is believed to involve glomerular filtration and tubular reabsorption(1,2). Giebisch *et al.*(1) suggested on the basis of the failure of water diuresis to cause increased ioduresis that 95% of filtered iodide is reabsorbed at a tubular site proximal to that of facultative water reabsorption. The stop flow technic of Malvin *et al.*(3) has offered a more direct means to localize the site of reabsorption of this anion.

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