2- to 4-fold enhancement of hemagglutinin titer. The hemagglutinating activity of the treated antigen was stable on freezing and on storage for at least 3 months at 4°C. Sera from children following vaccination with Jeryl Lynn strain live mumps virus vaccine or following natural mumps infection nearly always gave higher antibody titers when the tween-ether treated antigen was used in the HI test than when whole virus antigen was employed. The serum neutralization test, however, was more sensitive than the HI technique and was required to detect antibody in specimens with low level of antibody. Comparative titer values of sera obtained by the various test procedures are presented.

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Serum Complement: Changes After Skin Grafting in Guinea Pigs.* (32279)

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The role of complement (C′) in the rejection of tissue or organ transplants is unclear. However, this group of heat labile serum proteins, interacting with specific antibody in the presence of calcium and magnesium ions, regularly participates in other immunological cytocidal reactions such as immune hemolysis(1) and the lysis of bacteria, protozoa, and tumor cells(2,3,4). Sequential attachment of C′ components to cell-membrane surfaces under these circumstances results in membrane injury and cellular death (5). Changes in the level of serum C′ may result from variations in the rates of either its synthesis or utilization. Such changes have been sought in a variety of experimental and clinical settings. Injection of guinea pigs with antigen-antibody complexes results in nearly complete removal of whole C′ from their serum for a period of several hours(6). A decrease in serum C′ activity accompanies anaphylactic shock in the same animal(7). The level of serum C′ in patients with certain "autoimmune" diseases, such as systemic lupus erythematosus and glomerulonephritis, is nearly always depressed during the acute phase of these illnesses(8,9,10). Such observations are usually interpreted as a reflection of C′ consumption during acute-phase immunological reactions.

Evidence for the participation of C′ in the rejection of tissue and organ transplants is mounting. Winn(11) has shown that C′ participates in the destruction of transplants

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of lymphoma cells. According to Govaerts (12), donor kidney cells are agglutinated when combined with lymphoid cells and serum from the recipient of a renal allograft, but the donor cells are not lysed until C′ is added to the mixture. Antibody eluted by Hager and colleagues(13) from homogenized rejecting canine renal allografts fixed C′ when combined with donor antigen. Horowitz and co-workers(14) observed C′-binding at sites in which gamma globulin was deposited in the blood vessels of rejecting canine renal allografts. Clark *et al*(15) have shown considerable uptake of C′ by rabbit heterografts transplanted to the neck of the dog while Snyder and colleagues(16) were able to prolong the life of renal xenografts in the dog by treatment with a complement inhibitor.

We report a carefully controlled study of the levels of whole C′ in the serum of 2 groups of skin-grafted guinea pigs: one group, bearing autografts, in which graft rejection did not take place and another group, bearing allografts, in which graft rejection invariably occurred. Serial estimations of whole C′ over a period of 2 weeks after grafting in this model show that absence of rejection is characterized by a sustained *rise* in serum C′, while rejection is accompanied by a sustained *fall* in this fraction of serum.

Materials and methods. Line bred American albino guinea pigs were used in all experiments. A single full-thickness fitted skin graft 11 mm in diameter was sutured to a bed prepared on the thorax of each recipient animal under pentobarbital anesthesia. In allografted cases, the transplanted skin came from unrelated guinea pigs obtained from a separate supplier in a geographical location different from our own. Compression dressings were applied, and the grafts inspected for the first time on the fourth day after grafting and daily thereafter. Any animal without a primary take at 4 days was excluded from the experiment. For each whole C′ determination 0.5 ml of blood was drawn by cardiac puncture. In 27 *autografted* cases, 20 animals had C′ determinations 1, 4, 6, 8, 11, 13, and 15 days after grafting, and 7 had measurements made 1, 3, 5, 7, 9, 13, and 15 days after grafting. In 31 *allografted* cases, 22 animals had measurements of whole C′ 1, 4, 6, 8, 11, 13, and 15 days after grafting and 9 had measurement made 1, 3, 5, 7, 9, 13, and 15 days after grafting. All animals had preoperative baseline determinations one day before grafting. Thus data on serum C′ levels were available preoperatively and almost daily postoperatively over a period of 2 weeks in both groups of animals. Complement activity was assayed using a hemolytic microtitration test system modified from Kabat and Mayer(17). Freshly drawn blood samples were allowed to clot for 30 minutes at room temperature. After clot retraction overnight at 4°C, the serum was separated and immediately stored at —70°C until tested. Sheep red blood cells were optimally sensitized with purified 7S hemolysin prepared in this laboratory. Serial dilutions of serum samples in gelatin-Veronal buffer containing magnesium and calcium ions were made in microtitration plates and the sensitized cells added. The hemolytic reaction was allowed to proceed with shaking for 60 minutes at 37°C. After cold centrifugation of the plates, the C′ titer for each sample was read as the reciprocal of the highest dilution yielding 50% hemolysis. Each estimation was run in duplicate using a serum of known titer as a control. Allografts were considered to be rejected when circulation in the grafts had stopped and before any sloughing had occurred. Median survival times were calculated according to the method of Litchfield(18) and the median C′ titers of each group were plotted separately on arithmetic graph paper for each day of the experiment.

Results. The median survival time of allogeneic skin grafts in these experiments was 7.5 ± 0.2 days. The median values of whole C′ in each group for each day are shown graphically in Fig. 1. Two distinct patterns of response clearly emerged. One day after grafting, and before any circulation between graft and bed had been established, the serum C′ levels rose and to the same extent in both groups. In the autografted group, without rejection, the levels remained *elevated* above normal, with a gradual decline to normal 11 days after grafting. In contrast, in the al-

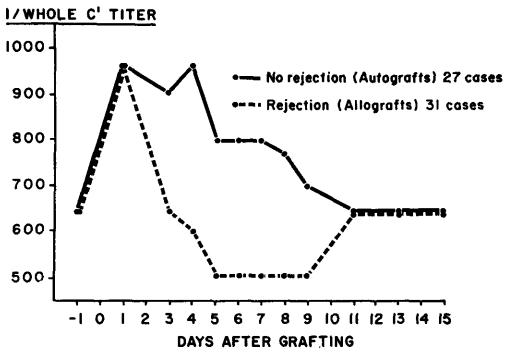

FIG. 1. Median levels of whole complement (C′) in serum of 2 groups of skin grafted guinea pigs, one without rejection (autografts) and one with rejection (allografts). C′ levels are consistently elevated in the autografted group and consistently depressed in the allografted group after the first postoperative day.

lografted group, with rejection, the level of whole C′ *fell* to normal 3 days after grafting, continued to fall and remained abnormally low until 11 days after grafting when a return to preoperative levels occurred. The period of sustained depression of serum C′ levels in the allografted group corresponded to the period of rejection manifested clinically. After circulation in the allografts had stopped, the serum C′ rose to normal levels. Table I correlates the changes in C′ levels in both groups with the biological and clinical events taking place. Without exception, the C′ levels in each animal in each group followed the pattern established for that group as a whole. For clarity the range of daily titers for each set of determinations has been omitted from the graph in Fig. 1. However, within each group of guinea pigs on each day, 75 to 85% of the values were clustered exactly at the median titer for that day. The results in a single allografted guinea pig, not included in these two series, are notable. Because of a chance histocompatibility, the allograft in this animal was never rejected and, at 15 days after grafting, had all the characteristics of a successful autograft. This was the *only* allografted animal in whom a depression of serum C′ did *not occur*. The pattern of response in this case corresponded exactly to that observed in autografted cases.

Discussion. We believe that the initial rise in serum C′ levels in both groups of guinea pigs and the sustained elevation in the autografted group are a non-specific response to the trauma of surgical operation. Such rises have been recorded after skin grafting in rats, after nephrectomy and adrenalectomy in dogs, and after splenectomy and myocardial infarction in man(19,20,21,22). The reasons for such rises in response to non-specific tissue damage and inflammation are unclear, but the fact of their occurrence makes the depression of serum C′ in those guinea pigs with rejecting allografts even more significant. Indeed Guiney(19) has commented that his failure to demonstrate a fall in titer in association with skin graft rejection in rats could have been due in part to masking by a rise in titer secondary to inflammation. He and his colleagues were able, however, to demonstrate significant depressions of the second component of human C′ in patients

TABLE I. Correlation of Changes in Levels of Serum Complement (C′) in Skin Grafted Guinea Pigs with Biological Events Occurring After Grafting.

Phase	Days after grafting	Biological events		Whole complement titer	
		Autograft	Allograft	Autograft	Allograft
I	1	Response to trauma	Response to trauma	Rises above normal	Rises above normal
II	2–4	Host-donor circulation established	Host-donor circulation established; microscopical rejection	Remains elevated above normal	Falls to normal or below
III	5–9	Early wound healing	Gross rejection	Remains elevated above normal; slow decline towards normal	Falls below normal
IV	10–15	Late wound healing	Host-donor circulation stops in all grafts	Falls to normal	Rises to normal

rejecting renal allografts. Our own clinical experience suggests that a fall in whole serum C′ may be found also in this setting.

We must stress that depressions of serum C′ do not, in themselves, prove that C′ is being bound in an ongoing immunological reaction. These data are correlative and only suggest that possibility, since a decreased rate of synthesis, decreased delivery into the circulating blood, or some other as yet unknown or nonspecific kind of C′ inactivation could give similar results. But apart from the biologically interesting differences observed in the two groups of animals which we report here, the practical implications of our results appear to be substantial. Such a consistent drop in serum C′ during rejection, in large numbers of animals, has not been reported previously. In addition, the depressions we observed occurred several days *before* rejection was clinically apparent. A reliable test for predicting the presence or absence of a rejection crisis is badly needed by clinicians who are performing organ transplants in man. Such a test could permit a step-up in immunosuppressive treatment before clinical rejection was at hand. The measurement of levels of whole serum C′ and C′ components may thus prove to be clinically useful.

Summary. The level of whole complement (C′) is consistently elevated postoperatively in the serum of guinea pigs receiving autografts of skin. In guinea pigs receiving allografts, the level of whole serum C′ is consistently depressed after the first postoperative day until rejection is completed. C′ may play a role in allograft rejection, and depressed levels of serum C′ may be a clue to clinical rejection.

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