ruptured cells may combine with antibody or with antibody-forming cells and thereby help in the establishment of a successful transplant, and 2) ruptured cells may induce the synthesis of antibodies which play a role in protecting the transplant from the SV40-induced immune response. The second explanation would be a type of immunological enhancement.

Summary. Newborn hamsters inoculated at birth with ghosts prepared from cells transformed by papovavirus SV40 and subsequently vaccinated as weanlings with the virus, failed to reject later challenge of the autologous tumor cells. In other experiments, the establishment of transplantation immunity conferred by inoculation of the virus into normal weanling hamsters could be interrupted with tumor cell extracts injected during the course of virus immunization. Once immunity had been established, however, inoculation of ruptured tumor cells failed to significantly alter the immune status of the animals.

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Effects of Conditioning upon Stress Responses in the Rat.* (31559)

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In a study(1) of the role of histamine-mediation of pituitary-adrenal activation by a variety of drugs and other stressful stimuli such as cold exposure in the rat, the problem of obtaining stable control values for plasma corticosterone levels was evident. The widespread use of stress as a tool for studying induction of enzyme synthesis and conclusions regarding enzyme regulation drawn therefrom, suggested the desirability of a controlled study to determine optimal methods of animal handling during such studies. Adaptation to handling and intravenous injection of saline was assessed by measuring plasma corticosterone elevation caused by subsequently administered histamine or saline. The combined effect of housing and conditioning to injection

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TABLE I. Effect of Saline Pretreatment on Plasma Corticosterone Levels in the Rat.*

Group	Treatment	Plasma corticosterone (μg/100 ml $\pm$ S.E.‡)
Saline-pretreated	a) Saline	24.5 $\pm$ 3.9 (11)†
	b) Histamine	66.0 $\pm$ 6.1 (11)
Control	c) Saline	46.6 $\pm$ 6.3 (5)
	d) Histamine	62.6 $\pm$ 2.7 (5)

* Rats were pretreated for 5 days with saline (1 ml/kg, i.v.) and on the sixth day received either saline (1 ml/kg) or histamine (5 mg/kg) intravenously, as did non-pretreated controls. The animals were sacrificed 30 min later and plasma taken for corticosterone analysis.

† Animals per group.

‡ Standard error. Level of significance: a *vs* c, $p < 0.001$; b *vs* d, $p > 0.6$.

was also evaluated in view of the report(2) that resting levels of plasma corticosterone in rats are influenced by the number of animals maintained per cage. Variation in resting levels of plasma corticosterone and adrenal ascorbic acid was measured over a 12-month period since recent reports(3) show that susceptibility of the rat to various stressors appears to be seasonally-dependent.

Methods and materials. Male rats of the Holtzman strain weighing between 180 and 280 g were used throughout this study and were maintained on commercial laboratory chow and water. Animals were held for 7 days after receipt from the supplier before use in stress studies and were housed at 76°F with alternating 12-hour periods of light and darkness. Drugs were administered and the animals sacrificed in the animal quarters between 8:30 A.M. and 11:30 A.M. Animals were sacrificed by decapitation within 30 seconds of removal from the cage. Free-flowing blood was collected in tubes containing a few mg of potassium oxalate, centrifuged to remove red cells, and the plasma frozen and stored until assayed for plasma corticosterone. The procedure of Guillemin *et al*(4) was employed in determination of plasma corticosterone.

The method described by Maickel(5) was used for determination of adrenal ascorbic acid. Adrenal glands were removed at sacrifice, trimmed of adhering fat and frozen until analyzed.

In the conditioning regimen animals received saline (1 ml/kg, i.v.) for 5 days prior to measurement of plasma corticosterone after saline or histamine injection (5 mg/kg, i.v., free base; administered as the diphosphate salt) on the sixth day. Rats were sacrificed 30 minutes after drug administration and plasma collected as described.

Other groups of animals were housed either individually or in groups of 10 for 8 days. From the fourth through the eighth day these animals were pretreated with saline as above. On the ninth day, rats from each group received saline (1 ml/kg, i.v.) or histamine (5 mg/kg, i.v.). Animals were sacrificed 15 minutes following administration of saline or histamine and plasma corticosterone levels determined as described.

Measurement of resting levels of plasma corticosterone and adrenal ascorbic acid was made on groups of from 3 to 24 rats during a 12-month period. These animals were not conditioned by saline injection nor were they handled prior to sacrifice. Care was taken that such animals were stunned and decapitated within 30 seconds of remvoal from community cages.

Results. Data presented in Table I indicate that conditioning of rats to intravenous saline administration inhibits the marked elevation of plasma corticosterone induced by saline in unconditioned animals. It should also be noted that the elevation of plasma

TABLE II. Plasma Corticosterone Elevation by Histamine or Saline in Rats Housed Individually or in Community Cages.*

Group	Treatment	Plasma corticosterone (μg/100 ml $\pm$ S.E.‡)
Isolated	a) Saline	35.7 $\pm$ 2.8 (6)†
	b) Histamine	58.3 $\pm$ 3.7 (6)
Community cages	c) Saline	32.3 $\pm$ 3.9 (7)
	d) Histamine	50.1 $\pm$ 3.0 (7)

* Animals were housed in individual or community (10) cages for 8 days. From the fourth through the 8th day all animals received saline (1 ml/kg, i.v.). On the 9th day animals from each group received saline (1 ml/kg) or histamine (5 mg/kg) intravenously and were sacrificed 15 min later for plasma corticosterone determination.

† Animals per group.

‡ Standard error. Level of significance: a *vs* c, $p > 0.6$; b *vs* d, $p > 0.1$.

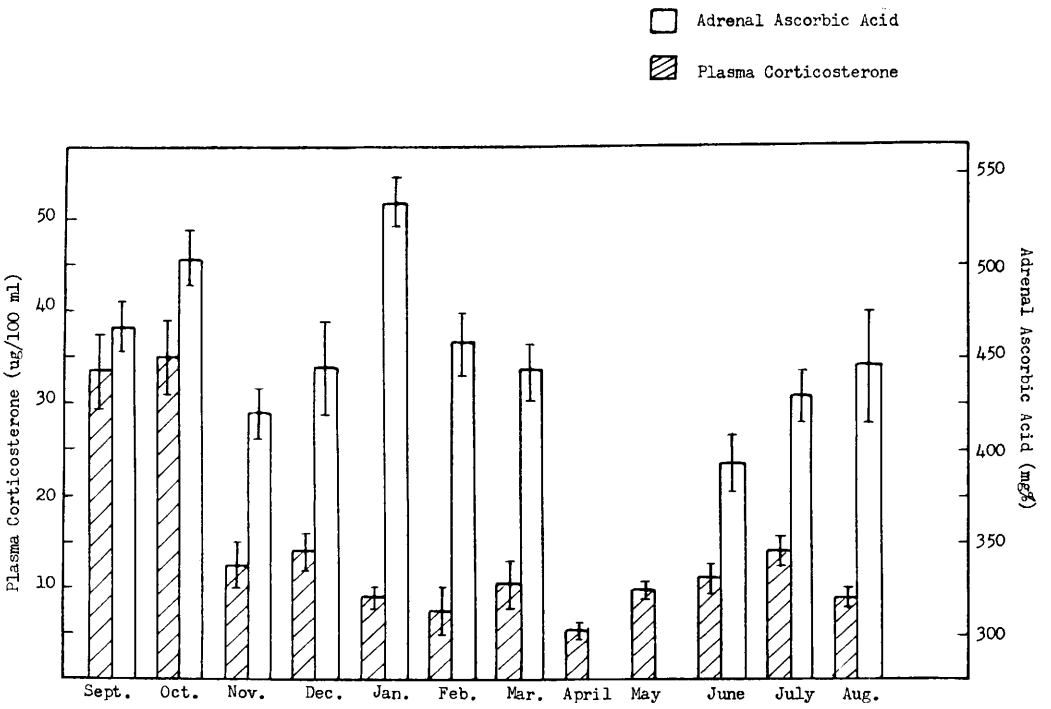

FIG. 1. Resting Levels of Plasma Corticosterone and Adrenal Ascorbic Acid in the Rat.

corticosterone produced by a potent stressor such as histamine is not influenced by prior administration of saline for 5 days.

Plasma corticosterone elevation produced by saline or histamine was not significantly different in rats housed individually or in community cages when both groups were conditioned to intravenous saline administration for 5 days (Table II). It thus appears that the reported(2) differences in resting levels of plasma corticosterone in rats housed under different group conditions is not reflected in the response of such animals to stressors, when both groups are adapted to handling and injection.

The mean value for resting levels of plasma corticosterone in 100 rats was found to be 11.3 $\pm$ 0.65 μg/100 ml plasma, which corresponds well with the value of 11.9 $\pm$ 3.9 μg/100 ml plasma reported by Knigge and Hoar(6). The corresponding value for adrenal ascorbic acid in 60 animals was found to be 447 $\pm$ 9 mg%.

Fig. 1 represents resting plasma corticosterone and adrenal ascorbic acid levels determined over a 12-month period (Sept. 1964-Aug. 1965). With the exception of high corticosterone levels in September and October, no consistent pattern of variation was noted. Basal levels of adrenal ascorbic acid vary more than those of plasma corticosterone and the assumed relationship between plasma corticosterone and adrenal ascorbic acid is not evident. The ratio of plasma corticosterone to adrenal ascorbic acid shows large fluctuations over the time period studied, which suggests that caution be employed in relying upon adrenal ascorbic acid determinations as an index of adrenocortical activity or response to stressors.

Discussion. The marked elevation of plasma corticosterone levels in the rat following saline administration suggests the advisability of conditioning animals to drug administration prior to their use in stress studies. While plasma corticosterone elevation produced by histamine is unaffected by conditioning (66.0 $\pm$ 6.1 *vs* 62.6 $\pm$ 2.7 μg/100 ml, $p>0.6$), there exists a highly significant ($p<0.001$) difference between the "saline controls." It is evident that experiments undertaken to quantitate plasma corticosterone elevation in

response to stressors would be markedly influenced by this factor. This point is of special significance in studies of enzyme induction by stressful stimuli.

Any influence of housing conditions on the plasma corticosterone elevation produced by histamine or saline administration can be prevented by conditioning to intravenous saline administration.

In general, a small variation was noted in resting levels of plasma corticosterone in the male rat over a 12-month period, while the variation in adrenal ascorbic acid levels was much greater. This observation leads us to question the reliability of adrenal ascorbic acid depletion as an indicator of adrenocortical response to stressors. In other experiments(1) we have noted further discrepancies in the relationship of adrenal ascorbic acid depletion and plasma corticosterone elevation in response to stressors. This relationship has been questioned by other authors(7,8).

It therefore appears that great care must be taken in all studies of adrenocortical response to stressors to ensure: (a) that basal or resting levels of plasma corticosterone are relatively constant in the population of experimental animals employed, and (b) that animals employed as "controls" be refractive to handling and drug administration.

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Potentiation of Purified Erythropoietin with Serum Proteins. II: Serial Dose Response Relationships.* (31560)

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The relationship of plasma proteins to erythropoietic activity has been the subject of much interest(1,2). We have reported the enhancement of erythropoietically active urinary fraction II + III(3) by normal human, rabbit and mouse sera and non-neutralizing antisera and suggested that the enhancing protein either a) provides a protective carrier, b) neutralizes an inhibitor of erythropoietin or c) activates an erythropoietin precursor (4). Recently Garcia and Schooley have shown enhancement of erythropoietin by normal serum when the material was injected subcutaneously into the test animals. However, this did not exceed the activity of a comparable amount of saline suspended erythropoietin given in divided doses subcutaneously(5). They suggested that the apparent enhancement of activity by the normal serum may actually be merely a reflection of slower and more prolonged absorption from the subcutaneous tissues when serum is added. The following experiments were carried out to evaluate this suggestion.

Materials and methods. The erythropoietically active human urinary concentrate (fraction II + III) was prepared by DEAE cellulose column chromatography(3). One milligram of this material is approximately equal to 0.2 unit of Erythropoietin Standard B (Medical Research Council, National Insti-

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