ever, the amount of phage in the blood and viscera following intraperitoneal injection was not significantly affected by treatment with endotoxin.

Ecologically, endotoxin may be an important controlling factor in antibody production. Endotoxin derived from the enteric bacteria, like orally administered actinophage (12) may be adsorbed in sufficient quantity to alter the antibody content of the sera(13).

Summary. Repeated injections of 4 mg endotoxin/kg, subsequent to antigenic stimulation with actinophage MSP8, retarded the early and late immune response in the mouse and resulted in an accelerated loss of preformed antibody. Cessation of endotoxin treatment permitted phage neutralizing activity in stimulated animals to increase.

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Effect of Guanethidine and Adrenal Demedullation on Hyperglycemic Responses to Diazoxide in Rats.* (29641)

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Diazoxide (7-chloro-3-methyl-1,2,4,-benzothiadiazine-1, 1-dioxide), a non-diuretic benzothiadiazine, is an effective hypotensive drug in animals(1-3) and in man(4,5). Unfortunately it produces significant hyperglycemia in normal, and especially, in diabetic subjects(6). The mechanism and the locus of action of this hyperglycemic effect are unknown. Diazoxide causes hyperglycemia in adrenalectomized, nephrectomized, or propylthiouracil-treated mice(7). Tolbutamide or insulin given to normal mice prevent or reverse the hyperglycemia(7,8). Adrenalectomy, alone or together with the administration of isopropyl methoxamine, a beta adrenergic blocker, or pretreatment with chlorisondamine, a ganglionic blocker, modify the response(7,9). These findings suggest that the metabolic effects of diazoxide are at least partly mediated by adrenergic hormones. The present studies were undertaken to see whether guanethidine, a peripheral depleter of catecholamines, and adrenal demedullation would suppress the hyperglycemic responses of rats to diazoxide.

Materials and methods. The experiments were done on 42 rats of the Long-Evans strain, weighing approximately 250 g each. The rats were fed Purina Laboratory Chow and water *ad libitum*. The effects of single injections of guanethidine, diazoxide, or both, on blood sugar and liver glycogen were measured in nonfasted normal rats and also in nonfasted rats which had surgical ablation of the adrenal medulla 2 weeks previously (Table I). Five normal rats and 5 demedul-

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TABLE I. Effects of Guanethidine and Diazoxide, Singly and in Combination, on Blood Glucose and Liver Glycogen of Normal and of Demedullated Rats.

	Rat group	No. of rats	Drugs	Blood glucose, mg/100 ml*	Liver glycogen, %*
A. Normal rats	I	5	None	102 ± 2.1	5.05 ± .2
	II	4	Guanethidine	96 ± 2.3	4.35 ± .4
	III	8	Diazoxide	540 ± 61.1†‡	1.47 ± .2†
	IV	5	Diazoxide and guanethidine	571 ± 27.2†	.35 ± .1†
B. Demedullated rats	V	5	None	102 ± 1.3	3.04 ± .3
	VI	4	Guanethidine	103 ± 4.1	3.01 ± .2
	VII	7	Diazoxide	500 ± 17.6†§	.83 ± .1†
	VIII	4	Diazoxide and guanethidine	217 ± 35.3†‖¶	.92 ± .3†

* Mean value ± S.E.

† Mean value is significantly different from control value (no drugs) at 1% level or better.

‡ The control mean blood sugar for these 8 rats before diazoxide was 115 ± 5.2 mg/100 ml; it was not significantly different from the mean blood sugar of control group (I).

§ The control mean blood sugar for these 7 rats before diazoxide was 100 ± 2.8 mg/100 ml; it was not significantly different from the mean blood sugar of control group (V).

‖ The control mean blood sugar for these 4 rats was 100 ± 3.4 mg/100 ml; it was not significantly different from that of control group (V).

¶ Mean values underlined in demedullated groups are significantly different from mean values of the corresponding treatment group of normal rats.

lated rats received no drugs and served as controls.

Guanethidine, 20 mg/kg, was injected subcutaneously in 4 normal and in 4 demedullated rats. Diazoxide, 200 mg/kg was given intraperitoneally to 8 normal and to 7 demedullated rats. Five normal and 4 demedullated rats received a subcutaneous injection of guanethidine, 20 mg/kg, 4 hours before diazoxide was given. The diazoxide solution was adjusted to a pH of 11.0 to 11.5 with a minimum amount of 1 N sodium hydroxide.

Blood sugars were measured by Nelson's modification of Somogyi's method and were obtained 7 hours after guanethidine and 3 hours after injection of diazoxide. Control blood sugar levels were also obtained in the normal and demedullated rats given diazoxide. After the final blood samples were obtained the rats were sacrificed immediately and liver glycogen was determined by the method of Montgomery(10).

Student's t-test was used for statistical comparison of the effects of the drugs on blood sugar and liver glycogen. In this study a P value of <0.05 was the criterion for statistical significance.

Results. The effects of guanethidine and diazoxide, singly, and in combination, on blood glucose and liver glycogen of normal and of demedullated rats are shown in Table I. Mean values ± 1 standard error are given.

Guanethidine alone had no appreciable effect on blood sugar in any of the rats. Before diazoxide was given the mean control blood sugars of normal rats (Group III) or of demedullated rats (Group VII) were not significantly different from those of their respective control groups given no drugs (I and V). Diazoxide produced a striking increase in blood sugar in both normal and demedullated rats 3 hours after injection. In this study, however, adrenal demedullation *per se*, did not significantly alleviate the hyperglycemic response to diazoxide.

When guanethidine was given to demedullated rats 4 hours before injection of diazoxide, the blood sugar increased from 100 ± 3.4 to 217 ± 35.3 mg/100 ml ($P<0.02$). This hyperglycemic response, however, was significantly less than that observed following administration of guanethidine and diazoxide to normal rats ($P<0.001$). The increase in blood sugar was also less than that seen in demedullated rats given diazoxide alone ($P<0.001$).

The mean liver glycogen of normal rats receiving guanethidine alone was not appreciably lower than that of the control group. When diazoxide was given to normal rats

(III) the liver glycogen content was markedly reduced. When diazoxide and guanethidine were given together liver glycogen was significantly lower than in any of the other 3 groups of normal rats.

In the demedullated rats the liver glycogen content of the group given only guanethidine (VI) was not appreciably different from that of the control group (V). Diazoxide alone produced a marked decrease in liver glycogen and the level was significantly less than that of Group V or VI. The average hepatic glycogen of the group receiving both drugs (VIII) likewise was markedly lower than that of group V or VI but not significantly different from the liver glycogen level of the group receiving diazoxide alone (VII).

The liver glycogen of the control group of demedullated rats (V) was lower than that of normal rats receiving no drugs (I). Guanethidine also produced a significant reduction in liver glycogen in demedullated rats (VI) as compared to their normal counterpart (II). Likewise diazoxide produced a small but statistically significant decrease in liver glycogen in the demedullated rats as compared to its effect in normal rats. Adrenal demedullation did not attenuate the decrease in liver glycogen following the combination of drugs.

Discussion. These data support the view that the metabolic effects of diazoxide are at least partly mediated by adrenergic hormones. Other studies indicate that diazoxide intensifies hyperglycemia induced by epinephrine administration and also that it produces appreciable increases in plasma free fatty acids in dogs(7). Similar lipid changes have been recently described in hypertensive patients who received diazoxide together with a diuretic benzothiadiazine(11). Isopropyl methoxamine, a specific blocker of the metabolic effects of epinephrine, attenuates diazoxide-induced augmentation of free fatty acids in dogs(7).

The control group of rats which had adrenal demedullations had a significantly lower mean liver glycogen content than that of the control group of normal rats. We cannot readily explain this discrepancy by a difference in body weight or in food intake after demedullation. The acute effect of the surgical procedure does not seem to be an important factor since 2 weeks elapsed before the liver glycogen measurements were made.

There are several possible reasons why the combined approach of adrenal demedullation and guanethidine failed to suppress completely the hyperglycemic response to diazoxide. First, guanethidine may not have produced adequate adrenergic blockade, although it markedly reduces the concentration of administered dl-7-H^3-norepinephrine in the heart and spleen, but not in the adrenal gland(12). Secondly the adrenal demedullation could have been incomplete. In this study, however, the completeness of demedullation was verified by thorough histologic examination. The possibility still exists that chromaffin tissue in other areas might have released catecholamines not blocked by guanethidine. Finally, the diabetogenic properties of diazoxide might be caused by one or more different mechanisms, perhaps unrelated to the adrenergic system. Direct toxic effects of diazoxide on the beta cells of the islets of Langerhans were not seen in the present studies and have not been described by other workers. The hyperglycemia is responsive to insulin, or, in patients with normal glucose tolerance, merely to cessation of diazoxide therapy. Increased insulin binding is apparently not a contributing factor(13).

Summary. A single intraperitoneal injection of diazoxide, 200 mg/kg, produced a significant elevation of blood sugar and a significant decrease in liver glycogen 3 hours after administration to normal and to demedullated rats. Guanethidine, 20 mg/kg subcutaneously, had no appreciable effect on blood sugar or liver glycogen of either group. When given together, diazoxide and guanethidine also produced a marked elevation in blood sugar and a significant reduction in hepatic glycogen in normal rats. The hyperglycemic effect of diazoxide was suppressed in the demedullated rats receiving guanethidine, although its depleting effect on hepatic glycogen was not attenuated.

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Tumor Immunity in Hamsters Infected with Adenovirus Type 12 or Simian Virus 40. (29642)

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It has been established that either adenovirus type 12(1) or simian virus 40 (SV_{40})(2,3,4) will induce neoplasms when injected into newborn hamsters. The minimum oncogenic dose of adenovirus type 12 for newborn hamsters as determined in cultures derived from human embryonic lung (5) is reported to be between 0.5 and 50 tissue culture infective doses ($TCID_{100}$). The minimum oncogenic dose of SV_{40} as determined by cytopathic changes in cercopithecus monkey kidney cell cultures is large. More than $10^{3.5}$ $TCID_{50}$ per 0.2 ml(3,6) was necessary to induce neoplasms in nearly every hamster, although smaller doses of virus sometimes caused tumor development in a few hamsters after a prolonged latent period.

Recently, it has been shown that the incidence of neoplasms in hamsters could be reduced or completely inhibited if additional doses of homologous virus were administered sometime after the initial infecting virus dose but before the appearance of tumors(7). The extent of the protection from tumor development varied from partial to complete. The experiments herein reported were designed to determine the effect of administering repeated large doses of virus to hamsters to determine the relationship between the degree of observed protection against tumor development with: 1) the initial infecting virus dose; 2) size of each virus dose used in treatment; and 3) total amount of virus given. The effect of the injection of repeated large doses of virus to normal hamsters beginning at 11 to 15 days of age was also tested. Except in the instance when incompletely inactivated SV_{40} was employed, all treatment was with undiluted fully virulent virus of the same lot which had been used to infect the animals when newborn, or of a similar lot.

Materials and methods. Viruses. Human adenovirus type 12, strain Huie, was obtained through the courtesy of Dr. M. J. Cook, Nat. Inst. of Allergy and Infectious Diseases. The virus was propagated in a continuous line of human carcinoma cells, HeLa(8), and the first virus lots prepared had titers of approximately $10^{2.0}$ to $10^{2.5}$ per 0.2 ml in 7 days. After several passages in HeLa cells and a