immune response to certain antigens may be, it is clear from the observations reported here that they interfere non-specifically with the productive phase of antibody formation rather than with the establishment of immunologic memory.

Summary. Lipopolysaccharide and its lipoid A component, when injected together with the common antigen of Gram-positive bacteria obtained from *S. aureus,* inhibit the production of circulating antibodies detected by hemagglutination. Nonetheless, the animals so treated are immunologically primed, as evident from a more rapid development of antibodies following a booster injection of antigen than is observed in unprimed animals. The common antigen of Gram-negative bacteria neither primes the animals nor functions as a booster antigen for the production of antibodies against the staphylococcal antigen, indicating the specificity of priming. The specificity of the immune response to staphylococcal antigen was ascertained by the fact that the antibodies react with erythrocytes modified by *B. subtilis* antigen as well, and that the latter antigen inhibits completely the hemagglutination of red blood cells modified by staphylococcal antigen. It is postulated that lipopolysaccharide (endotoxin) and its lipoid A component interact with certain antigens *in vitro,* and that the inhibitor, when injected into rabbits together with the antigen, interferes with the production of circulating antibodies without eliminating the establishment of immunologic memory.

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Insulin Secretion *in vitro* by Pancreatic Tissue from Normal, Adrenalectomized, and Cortisol-Treated Rats.* (31887)

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Decreased tolerance to glucose is a classical feature of hyperadreno-corticism(1,2) and has been related to the antagonistic effects of glucocorticoid and insulin upon hepatic (3) and peripheral(4) glucose metabolism. The impaired tolerance to glucose occurs despite hyperplasia of the Islets of Langerhans (5,6,7) and elevated levels of circulating insulin(8,9,10). A possible explanation for

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hyperactivity of the beta-cells in hyperadrenocorticism was sought in the present study of insulin secretion induced *in vitro* by glucose with pancreatic tissue from normal, adrenalectomized, and cortisol-treated rats.

Materials and methods. Rats. Three groups of albino rats (150-250 g; Holtzman, Wis.) were studied. *Group A.* After bilateral adrenalectomy, 8 rats received daily subcutaneous injections of cortisol (2 mg; Hydro-cortisone, Sigma Chemical Co., St. Louis, Mo.) suspended in saline, and 8 received no treatment. Sham operations, involving visualization but not removal of the adrenal glands, were carried out on 7 other animals. *Group B.* Of 15 animals completely adrenalectomized, 7 were treated with cortisol; 6 other rats were sham operated. *Group C.* Of 18 normal rats, 6 received injections of cortisol (5 mg/day) for 5 days and 6 for 2 days; the remaining 6 received no treatment. All animals had free access to food (Groups A and B, Wayne Lab Blox, Allied Mills Inc., Chicago; Group C, Lab Chow, Ralston Purina Co., St. Louis, Mo.) and either water or saline (adrenalectomized and sham operated rats). Adrenalectomized and sham operated rats were killed 3 days after surgery, and normal animals after 2 or 5 days of treatment with cortisol. A daily check of body weight was kept during the period of treatment.

On decapitation, blood was collected and the plasma separated for sugar estimation with the Autoanalyzer (Technicon Instruments Co., Chauncey, N. Y.) by a method based on that of Hoffman(11). The pancreas was removed and used for incubation (Group A), insulin extraction (Group B) or both (Group C).

Insulin secretion. Small pieces of pancreas (*ca* 10 mg each) in groups of 4 were placed into bicarbonate-buffered media (2 ml) containing glucose (150 mg/100 ml), bovine serum albumin (0.5%, w/v; Bovine albumin, Fraction V, Sigma Chemical Co., St. Louis, Mo.) and guinea pig anti-insulin serum (GPAIS, Lot Nos. 270 and 335). Sufficient GPAIS was added to the medium to bind about twice the expected amount of secreted hormone, insulin secretion over 90 minutes of incubation at 36°C being equated with the fall during incubation in insulin antibody content of the medium. Full details of the method are described elsewhere(12,13).

Insulin content of the pancreas. Either the entire pancreas (*ca* 1.0 g; Group B) or small pieces dissected beforehand in iced buffer (*ca* 0.2 g/Group C) were homogenized in acid-alcohol (5 ml; ethanol, 96 vol, conc H_2SO_4, 2.4 vol, and water, 18 vol) and then further diluted with the same solution of acid-alcohol either to 50 ml (entire pancreas) or to 10 ml (pieces). After subsequent centrifugation, an aliquot (10 vol) of supernatant solution was neutralized with sodium bicarbonate (8 vol; 16%, w/v) and made up to volume (20 vol) with a buffered solution of albumin (1% bovine albumin in phosphate buffer, 0.1 M, pH 7.0).

Aliquots of this neutralized extract (0.2 ml) were incubated for 60 minutes at 36°C with a constant volume (10 μl) of GPAIS (Lot 380, binding about 2.5 mU insulin/μl) diluted in the buffered solution of albumin (0.8 ml). From the loss of insulin binding capacity of this GPAIS and those of the same volume of GPAIS incubated with varying amounts of rat insulin (0-10 mU), the insulin content of the neutralized extract and of the original pancreatic tissue were calculated. Details of the method for assay of partially neutralized GPAIS have been described elsewhere(14).

Mean rates of insulin secretion and the mean insulin content of pancreatic tissue are here reported in relation to the amounts of incubated (uU/mg wet wt/90 min) or extracted (mU/mg) tissue.

Results. As shown in Table I, slight decreases in body weight (*ca* 5%) and plasma sugar concentration occurred during the 3 days following adrenalectomy, loss of body weight also being observed in adrenalectomized rats treated with cortisol. Mean pancreatic weight and mean insulin content of pancreatic tissue were unaffected by adrenalectomy or by subsequent treatment with cortisol. By contrast, adrenalectomy caused a marked reduction (*ca* 40%, $p<0.001$) in secretion of insulin which could be provoked by glucose in pancreatic tissue *in vitro,* an effect which was not found with tissue from

TABLE I. Effect of Adrenalectomy on Insulin Secretion. For each experimental condition, the Table indicates body weight (group A and B; see *Material and methods*) at time of surgery (day 0) and death (day 3); level of plasma sugar at death (group B); weight and insulin content of pancreas (group B); and insulin output measured *in vitro* (group A). Mean values (± S.E.M.) are shown together with number of determinations (in parentheses).

	Sham operated	Adrenalectomized	Adrenalectomized & cortisol, 2 mg/d
Body wt (g)			
Day 0	219 ± 8 (13)	217 ± 9 (15)	213 ± 8 (15)
" 3	215 ± 5 (13)	206 ± 7 (15)	196 ± 7 (15)
Plasma sugar (mg/100 ml)	151 ± 3 (6)	131 ± 5 (8)	141 ± 3 (7)
Pancreas			
Weight (mg)	1138 ±51 (6)	1075 ±35 (8)	1113 ±33 (7)
Insulin (mU/mg)	2.21 ± .08 (6)	2.24 ± .10 (8)	1.93 ± .16 (7)
Insulin output (μU/mg/90 min)	40.2 ± 2.1 (63)	24.8 ± 1.5 (72)	44.4 ± 1.9 (72)

TABLE II. Effect of Cortisol Administration on Insulin Secretion. Cortisol administration was initiated 5 days (third column) or 2 days (second column) before death. The Table indicates mean values (± S.E.M.) for body weight before (day 0) and during (day 3) treatment, and at death (day 5); level of plasma sugar at death; weight and insulin content of pancreas; and insulin output provoked by glucose *in vitro*. Number of determinations is shown in parenthesis.

		Cortisol, 5 mg/d	
	Control	2 days	5 days
Body wt (g)			
Day 0	229 ± 5 (6)	227 ± 4 (6)	226 ± 4 (6)
" 3	252 ± 6 (6)	251 ± 5 (6)	202 ± 3 (6)
" 5	268 ± 6 (6)	234 ± 3 (6)	189 ± 3 (6)
Plasma sugar (mg/100 ml)	150 ± 3 (6)	159 ± 5 (6)	164 ± 7 (6)
Pancreas			
Weight (mg)	1020 ±55 (6)	851 ±38 (6)	789 ±22 (6)
Insulin (mU/mg)	1.74 ± .17 (6)	1.38 ± .13 (6)	1.66 ± .23 (6)
Insulin output (μU/mg/90 min)	36.4 ± 2.2 (54)	50.4 ± 2.8 (54)	53.6 ± 2.9 (54)

adrenalectomized rats treated with cortisol.

When normal rats were treated with cortisol (Table II), there was progressive loss of body weight over 5 days, a slight increase in plasma sugar concentration, and a minor decrease in the insulin content of the pancreas. Compared with insulin secretion provoked by glucose in pancreatic tissue of normal rats, that of tissue from rats treated with cortisol was markedly increased (+ 40 to + 50%, $p<0.001$).

When Methylprednisolone (5×10^{-5} M; Solumedrol, Upjohn Co., Kalamazoo, Mich.) was incorporated in the incubation medium, insulin secretion provoked by glucose (150 mg/100 ml) was unaffected whether the incubated tissue came fom normal or adrenalectomized rats (Fig. 1).

Discussion. From the results of these experiments it can be concluded that cortisol, when administered *in vivo* for 2 to 5 days, increases the sensitivity of pancreatic islet tissue to glucose; but when added to incubation media *in vitro,* has no effect upon glucose-induced insulin secretion by pancreatic tissue from normal or adrenalectomized rats. By contrast, removal of the adrenal glands *in vivo* results in reduced responsiveness of the isolated pancreatic tissue to glucose within 3 days.

These alterations in the responsiveness of the pancreatic beta cells to glucose cannot be related to any accompanying changes in insulin content of the pancreas. In fact, a

marked increase in responsiveness to glucose was found in tissue from normal animals treated with cortisol, the only group in which a slight decrease in pancreatic insulin was found (Table II). It is also unlikely that the reduced responsiveness produced by adrenalectomy was due to fasting during the postoperative period. These animals did not lose significantly more weight than the sham-operated rats and food was found in their gastro-intestinal tracts at the time of death. Moreover, when treated with cortisol they lost even more weight but insulin secretion provoked by glucose in their pancreatic tissue was either normal or, in some cases, even greater than that of tissue from the sham-operated rats (Table I).

Such increased sensitivity of the insulin secretory mechanism to glucose would account for the elevated levels of circulating insulin found in patients and animals with hyperadrenocorticism and normal concentrations of glucose in the blood(9,10). The explanation of the phenomenon itself is unknown and the results of the present experiments do not suggest that it is due to any immediate effect of glucocorticoids upon the insulin secretory mechanism. These hormones have been shown to have immediate effects upon metabolic processes in the liver of the rat(15), and the epidermis of the mouse(16). Apart from these isolated instances in which effects of glucocorticoids have been demonstrated *in vitro,* cortisol is known to have a marked effect *in vivo* upon the synthesis of enzymes regulating glucose metabolism in the liver(17) and in the beta cells of the Islets of Langerhans(18). It is possible, therefore, that glucocorticoids could be directly responsible for changes in the sensitivity of the beta cells to glucose, but that such changes occur slowly and could not therefore be demonstrated during the short period of incubation used in the present experiments (90 min; Fig. 1).

Summary. Insulin secretion provoked by glucose in the pancreas of the rat is not modified by addition of methylprednisolone to the incubation medium. Secretion by pancreatic tissue of the normal rat is reduced (to 60%) by prior adrenalectomy and increased (by 50%) after treatment with cortisol for 2 or 5 days. Treatment with cortisol for 3 days prevents the effect of adrenalectomy. These alterations are not associated with significant changes in the insulin content of the pancreas, and suggest that glucocorticoids increase the sensitivity of the insulin secretory mechanism of the beta cells to glucose either directly or indirectly in a chronic process.

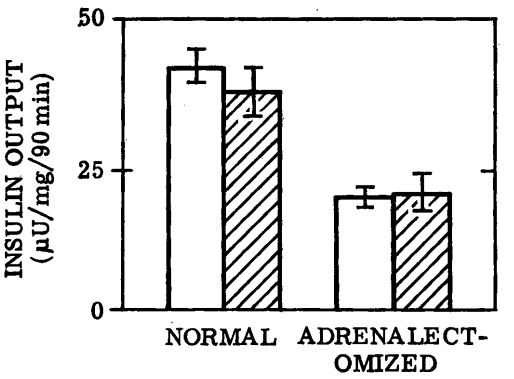

Fig. 1. Insulin output by pancreatic tissue removed from normal and adrenalectomized rats and incubated for 90 min, at a glucose concentration of 150 mg/100 ml, in presence (shaded columns) or absence (unshaded columns) of methylprednisolone (5.10^{-5}M). Each value represents mean (± S.E. M.) of 10 determinations.

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Cyclophosphamide Inhibition of Experimental Allergic Encephalomyelitis in Wistar Rats.* (31888)

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Prevention of auto-immune tissue injury by immunosuppressive drugs carries the promise of additional understanding and better control of harmful immunological responses of animals and man. This paper describes the suppression of experimental allergic encephalomyelitis (EAE)—a prototype auto-immune disease—in rats treated with cyclophosphamide.

Suppression of EAE has been reported by other workers using rabbits and/or guinea pigs treated with nitrogen mustard(1,2), 6-mercaptopurine(3,4), chlorambucil(5), methotrexate(6,7) or cyclophosphamide(7,8). In those animals observed after stopping the immunosuppressive agent in question, EAE usually appeared within a matter of 9 to 20 days, the usual latent period for this disease in rabbits and guinea pigs.

The present report describes inhibition of EAE in rats not only during administration of cyclophosphamide but for at least 3 weeks after discontinuing treatment with this alkylating agent. Cyclophosphamide administration beginning as late as 9 days after nervous tissue sensitization also inhibtis the disease. In addition, this alkylating agent has been found to inhibit production of antibrain antibody in parallel with inhibition of EAE.

Methods. Cyclophosphamide (Cytoxan®).‡ The drug was supplied in vials containing 100 mg dry powder and was stored at 4°-10°C until dissolved in sterile physiological saline and injected intraperitoneally into lightly etherized rats. In most experiments, cyclophosphamide was injected daily or 5 days a week beginning on day of sensitization and continued through the 17th post-sensitization day. Rats were weighed weekly to be sure the same dose of the drug per kilogram was given throughout each course of treatment. *Rats and sensitization procedure.* Female Wistar rats§ (125 to 250 g each) were used and maintained on commercial food pellets and water *ad lib.* Hartley strain guinea pig spinal cord aseptically removed and stored 1 to 17 days at —20°C was homogenized in 0.25% phenol in distilled water to give a 33% suspension and incorporated into an equal volume of Freund's complete adjuvant as previously described(9). Each rat was sensitized by injection of 0.1 ml spinal cord-adjuvant emulsion into each of 6 skin sites over the upper back and 1 skin site over the anterior neck(9). *EAE criteria:* Sensitized rats were observed closely for clinical neurological signs, these usually being ataxic gait and/or paraly-

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§ Female Wistar stock (CFN) rats purchased from Carworth, Inc., New City, New York.