TABLE I. Mice Dying from EO771 Homografts Taken from Single and from First or Second of Double Inoculations into an Isograft Host.

Exp.	Recipients strain	Sex	Tumor mortality according to Source of donor homograft: Single inoculation	First of double	Second of double	P
1*	BALB/c Jax		4/121		12/ 20	.01
2	"	♀	0/ 42	1/13	0/ 26	N.S.†
	"	♂	0/ 38	5/14	0/ 5	.01
3	"	♂	6/136	8/31	14/ 82	.01
	"	♀	2/149	2/20		.01
	C57BR/cd	♂	0/ 90	2/18		.01
	Sum‡ BALB/c		12/444	15/65	26/107	.01
			2.7%	23.1%	24.3%	

* First series of experiments(9).
† Donor tumor from a male with resulting low incidence among females(11).
‡ Values for male donor into female recipient (Exp. 2) omitted.

ploying 675 BALB/c, 108 C57BR/cd and 50 C57BL/6 mice, the first of double isografts of EO771 mammary carcinoma acquired an altered enhanced status comparable with that of the second of double isografts and each of the double isografts had 9 times the homotransplantability (24%) of single control isografts (2.7%). Within the conditions of the experiment neither size, nor age of the isograft was of importance. The altered enhanced status was abiding as determined by serial homotransplantability without further treatment.

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Nucleic Acid Levels of Rat Anterior Pituitary Glands Following Adrenalectomy.* (24771)

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The role of nucleic acids in protein synthesis has been reviewed by Brachet(1). Although endocrine organs are relatively poor in ribonucleic acid (RNA)(1), histochemical studies of the anterior pituitary gland demon-

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strate fluctuations in ribonucleoprotein corresponding to changes in secretory activity and related to protein hormone synthesis(2). Since results of histochemical methods do not lend themselves readily to quantitation, an investigation was undertaken to establish normal nucleic acid levels in the anterior pituitary of the rat and to demonstrate quantitative differences with procedures known to induce excessive hormone elaboration. This study utilizes surgical adrenalectomy as means of inducing increased protein hormone production from the pituitary. The literature contains ample evidence to substantiate the resultant increased pituitary adrenocorticotrophic hormone (ACTH) secretion following such procedure.

Methods. Male rats of Holtzman strain were bilaterally adrenalectomized *via* dorsal approach and maintained on Purina Laboratory Chow pellets and 1% NaCl *ad lib.* Unoperated controls received tap water in place of salt solution. At various times after operation(2, 3, 5 and 7 days), groups of adrenalectomized and control rats were sacrificed by decapitation and pituitary glands collected. Posterior lobe tissue was discarded and anterior lobes weighed on torsion balance and homogenized in normal saline to make a 0.6% homogenate. Determination of desoxyribose nucleic acid (DNA) and RNA from pituitary homogenates followed procedure described by Schneider(3).

Results. The data for pituitary nucleic acid levels of control and adrenalectomized rats are summarized in Table I. Calculated on the basis of μg of nucleic acid mg of anterior pituitary tissue, quantity of RNA in glands from control animals varied from 8 to 12 μg and range of values for DNA was 13-15 μg. Average ratio of RNA DNA was approximately 0.70. Two days after adrenalectomy, pituitary tissue contained 12-18 μg of RNA and 9-11 μg of DNA, an average ratio of about 1.50. Animals sacrificed 3, 5, and 7 days post-operatively contained pituitary RNA levels comparable to those of control rats and DNA contents of 10-11 μg/mg of tissue. These latter groups of operated rats demonstrated RNA/DNA ratios in the range of 0.70-0.90.

TABLE I. Pituitary Nucleic Acid Levels after Adrenalectomy.

Group	No. of rats	RNA μg/mg	DNA tissue	Avg RNA/DNA
Control	18	8–12	13–15	.71
Adrex—2 days	8	12–18	9–11	1.5
3 "	4	7	10	.7
5 "	4	10	11	.9
7 "	4	8	10	.8

Discussion. The choice of time following adrenalectomy to sacrifice animals was determined by reports in the literature indicating the time when pituitary ACTH elaboration was rapidly increasing. Gemzell *et al.*(4) reported that immediately after adrenalectomy there was a sharp drop in pituitary ACTH content, but after 24 hours the glandular content began to rise and eventually reached a level above normal in one week. The report of Fortier(5) is in general agreement with Gemzell as far as the 1- 7-day assays of pituitary ACTH are concerned, Therefore, the period from 2 to 7 days following adrenalectomy was selected for pituitary nucleic acid analyses, since rapid protein hormone synthesis must accompany increased ACTH elaboration. Our assay data support that premise. RNA/DNA ratio at 2-day post-adrenalectomy period was more than twice that observed in controls. Following this period of rapid protein hormone synthesis, as ACTH in the gland reaches a new level of production, nucleic acid ratios return to normal. Apparently the gland can produce hormone somewhat above normal amount without greatly accelerating protein synthesis during this time. Another explanation to account for the return to normal levels of protein synthesis might be based on the findings of Gemzell and Heijkenskjöld(6). Using exogenous ACTH they were able to decrease the pituitary content of that hormone in adrenalectomized rats in 2-3 weeks. Perhaps excessive *endogenous* ACTH produced by adrenalectomized rats has a similar depressing effect on protein hormone synthesis.

The apparent decrease in DNA/unit weight of gland with adrenalectomy probably reflects the relative increase of cytoplasmic elements of the gland brought about by hypertrophy

and hyperplasia of cells responsible for ACTH elaboration. Nevertheless, the difference in relative amounts of DNA between control and operated rats does not account entirely for larger nucleic acid ratio in the 2-day post-adrenalectomy group. The absolute increase in cytoplasmic RNA at that time is sufficient to overcome this relative DNA differential and indicate accelerated protein synthesis.

Summary. Adenohypophyses of adrenalectomized male rats were analyzed for RNA and DNA at various post-operative intervals. The ratio of RNA/DNA at 2 days after adrenalectomy was more than twice that observed in control animals. Rats sacrificed at 3, 5, and 7 days post-adrenalectomy revealed nucleic acid ratios comparable to those of controls. The increased RNA level in the pituitary 2 days after adrenalectomy correlates well with the time when glandular ACTH content is rapidly increasing. The increased elaboration of protein hormone ACTH induced by removal of adrenals can be correlated with alterations in nucleic acid levels reflecting increased protein synthesis.

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Zymosan as a Possible Reagent in Local Shwartzman Reaction. (24772)

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Recently, one of us (WTB) reported the tumor-necrotizing effect of yeast polysaccharide zymosan when administered to mice bearing implants of Sarcoma 180(1). Phenomena similar to this in which polysaccharide extracts of certain Gram-negative bacteria (endotoxin) produce hemorrhagic necrosis of tumors have been well documented in the literature(2,3,4). This action of bacterial endotoxins is thought by some to be a special form of localized Shwartzman reaction in which the tumor tissue is "naturally" prepared. Indeed, evidence supporting this view has been advanced by Shear and co-workers (5). Inasmuch as zymosan is a polysaccharide of microbial derivation(6), the possibility exists that its anti-tumor activity is due to endotoxin-like properties rather than to host-mediated immune phenomena postulated by Bradner *et al.*(7). It was our purpose to explore this possibility with tests designed to determine competence of zymosan in a system in which bacterial endotoxin plays a known part, namely, the localized Shwartzman reaction. It would appear that if tumor-necrotizing action of zymosan is due to "endotoxic" properties, its effectiveness as a preparing and/or provoking agent in the Shwartzman phenomenon should be readily demonstrable at concentrations active for tumor-necrosis. The dermal Shwartzman reaction, which was long undetected in mice has recently been demonstrated in selected mouse stocks(8,9). Inasmuch as the original observations of zymosan-induced tumor necrosis were made with mice, it seemed appropriate in so far as possible, to test the effects of this material in mice as well as in the classical system with rabbits.

Materials and methods. Mice were adult (20-24 g) females from either brother-sister inbred BSVS strain(10), or the Webster-Swiss strain (O'Grady Farms). Zymosan was

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