optimum, after which, excess antigen leads to a decrease in the cell reaction. A similar relationship was shown with lymph node cells by Oppenheim *et al*(6). The reduction of response *in vitro* by high doses of antigen resembles the induction of unresponsiveness in immunized animals(15,16), but may as well be due to toxic effects.

The mechanisms involved in the response of the rabbit lymphocyte *in vitro* and its relationship to other immune reactions are under investigation.

Summary. Lymphocytes from the blood of rabbits immunized with extracts of bovine or rat heart or with human IgG, reacted *in vitro* when cultured with the specific antigen. The reaction consisted of intense blast transformation, mitotic activity and incorporation of H^3-thymidine. The level of response was found to be dose-dependent. Some unstimulated (control) cultures showed moderate "spontaneous" blast formation, without, however, mitotic activity or thymidine uptake.

Thanks are due to Amos Shapiro for his continued assistance.

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Received May 18, 1967. P.S.E.B.M., 1967, v125.

Relationship of Life Maintaining Properties of Corticoids to Their Influence on Compensatory Ovarian Hypertrophy in Hemicastrated-Adrenalectomized Female Rats. (32343)

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Hypertrophy of the remaining ovary after hemicastration is a well-known phenomenon. Although the mechanism of the hypertrophy remains to be elucidated, we believe that removal of one ovary results in increased gonadotrophin secretion (synthesis and/or release) by the pituitary gland(1) which produces growth of the ovary. Adrenalectomy, at the time of unilateral ovariectomy, will abolish the ovarian response, while adrenal replacement therapy with glucocorticoids or mineralocorticoids or saline support will restore the ovarian response(2). At the original corticoid doses employed (cortisol, 1000 μg/day or DOC, 500 μg/day) most animals survived the 2-week experimental period, whereas mortality was high in the untreated groups. In an effort to determine the relationship of corticoid normalization of the ovarian response to their life maintaining effects, we have now examined series of doses of desoxycorticosterone and corticosterone in hemicastrated-adrenalectomized rats.

Materials and methods. Adult (160-180 g) female Charles River CD® rats were adrenalectomized and hemicastrated by removal of the right ovary. Drug treatment began immediately after operation and continued for

TABLE I. Effects of Varying Doses of Desoxycorticosterone (DOC) or Corticosterone on Survival and Ovarian Compensatory Hypertrophy of Adrenalectomized-Hemicastrated Rats. (I = intact; H = hemicastrated; Adx = adrenalectomized)

Compound	Daily dose (μg)	Condition	N	N Survived	% Survival	Left ovarian wt
Oil only		I-I	34	34	100	38.8 ± .91
" "		H-I	24	24	100	58.4 ± 2.06
DOC	1	H-Adx	10	8	80	41.7 ± 3.23†
	3	H-Adx	32	14	44*	39.6 ± 4.48†
	10	H-Adx	33	27	82*	43.7 ± 2.51†
	30	H-Adx	23	22	96	44.0 ± 2.75†
	100	H-Adx	24	24	100	45.8 ± 2.46†‡
	125	H-Adx	9	9	100	55.8 ± 3.16‡
	250	H-Adx	8	8	100	58.3 ± 3.60‡
	500	H-Adx	9	9	100	58.1 ± 2.71‡
Oil only		I-I	33	33	100	36.3 ± 1.09
" "		H-I	34	34	100	58.8 ± 1.59
Corticosterone	0.3	H-Adx	24	15	62*	40.8 ± 4.48†
	1	H-Adx	24	15	62*	46.7 ± 3.29†‡
	3	H-Adx	23	18	78*	45.7 ± 2.92†‡
	10	H-Adx	34	21	62*	48.1 ± 3.84†‡
	30	H-Adx	9	7	78	52.4 ± 7.70‡
	100	H-Adx	9	9	100	49.0 ± 3.83†‡
	125	H-Adx	10	10	100	49.8 ± 2.83†‡
	250	H-Adx	9	9	100	56.7 ± 2.03‡
	500	H-Adx	10	10	100	46.7 ± 2.82†‡

* $p < .05$ that observed figures are equivalent to 100% survival(4).
† $p < .05$ that observed values equal those of hemicastrated controls.
‡ $p < .05$ that observed values equal intact controls.

14 days. The rats were sacrificed for autopsy on the day following the final injection and the numbers of surviving animals noted. At autopsy the ovaries were cleaned of adherent tissues and weighed. Compounds were administered in corn oil solution and corn oil served for control injections.

Results. In each set of experiments, the control hemicastrated animals showed a satisfactory ovarian hyperthrophy over the intact controls (Table I), which confirmed results of earlier studies(1,2,3). We have not reëxamined the effects of adrenalectomy in this series of studies; however, the absence of an ovarian response at the low doses of the corticoids demonstrates that the response is abolished in conditions of adrenal insufficiency. Postoperative ovarian growth was essentially normalized in adrenalectomized rats at daily doses of 125 μg of DOC and above. The dose-response curve for DOC rose rapidly between the 100 and 125 μg doses; none of the values at this latter dose or above is significantly different from hemicastrated controls. The curve for corticosterone rose gradually, peaking at a dose of 250 μg and then falling slightly. Thus it is difficult to determine a dose for corticosterone that will normalize the ovarian response. Doses of 30 μg/day and above appeared to produce ovaries that approximate the size of the ovaries of hemicastrated rats with intact adrenals, although the entire series of data do not suggest a complete normalization of the response.

Adequate doses of both corticoids were almost entirely effective in maintaining life in treated animals; therefore, statistical significance of survival in individual groups was determined by comparing each group to an arbitrary standard of 100% survival. Of 32 rats at the 3 μg dose of DOC, only 14 survived, which is virtually identical to the survival (6 of 16 rats) reported earlier for unmaintained hemicastrated-adrenalectomized rats(2); the 80% survival for animals at 1 μg DOC is probably a statistical anomaly resulting from small numbers. At 10 μg of DOC survival was increased to 82%, which was significantly less than 100%. With 30 μg of corticosterone 78% of the rats survived, which was not significantly different from complete survival. Thus corticosterone and DOC both maintain essentially 100% survival at doses of 30 μg and higher.

Discussion. Results of DOC treatment indicate a clear separation between life maintenance and restoration of ovarian-compensatory-hypertrophy: 30 μg is almost completely effective in supporting life, whereas 4 or more times that amount is required to restore the ovarian growth effect. We have recently suggested(2) that the failure of adrenalectomized rats to respond to hemicastration resulted from the abnormal salt and water metabolism that follows adrenal removal. The present study may not be entirely commensurate with this hypothesis since a much lower dose is adequate to maintain life than is necessary to restore the ovarian response. Corticosterone administration also restores the ovarian response, although the data suggest only a partial restoration of the ovarian compensatory hyperthrophy.

Summary. Desoxycorticosterone will restore the ability of adrenalectomized rats to respond to hemicastration with ovarian compensatory hypertrophy; however complete restoration of the response was only obtained at doses 3-4 times those necessary to preserve life. Corticosterone-treated, adrenalectomized rats show a recovery of the ovarian response toward normal, but only at doses exceeding those necessary for life maintenance.

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Received May 22, 1967. P.S.E.B.M., 1967, v125.

Studies on Cardiac Glycogen in Normal and Thyroidectomized Rats.* (32344)

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Hypothyroidism in rats has been shown to increase the content of glycogen in the heart, but the mechanism responsible for this alteration has not been elucidated(1). From other studies it is known that cardiac glycogen is increased under circumstances of sustained fat mobilization(2,3) and is depleted by catecholamines(4). The present studies were designed to explore the effect of fasting and of catecholamines on the cardiac glycogen of normal and thyroidectomized rats.

Methods and materials. Male Holtzman rats were maintained on tap water and Purina Labena Chow for at least 4 weeks before study. Thyroidectomy was performed on 130 g rats as previously described(5) and hypothyroidism was assessed by failure of gain in weight(6). Any animal weighing more than 200 g 4 to 6 weeks after thyroidectomy was discarded; normal controls weighed between 250 and 400 g after the same length of time.

Experimental procedure. In experiments on fasting rats animals were deprived of food for 18 to 24 hours. The animals were anesthetized with pentobarbital and in some experiments blood was drawn from the abdominal aorta, following which the heart was promptly removed, weighed and dropped in 30% potassium hydroxide in a boiling water bath. Hearts were digested for at least one hour and analyzed for glycogen as previously described(7). Cardiac glycogen by this procedure was 5.8 ± .3 μg/mg and compared favorably with glycogen values of 5.0 ± .7 μg/mg determined on hearts frozen immediately after thoracotomy and before weighing. Free fatty acids were determined by the method of Dole(8) on 0.5 ml aliquots of plasma. Isoproterenol was given in a dose of 50 μg/kg subcutaneously at various intervals before death or in various doses by the

* This study was supported in part by Grants 2A-5166, A-612, and AM-09897 from Nat. Inst. Health.