present case antibiotics) may change the specific gravity of the tissues in addition to the change in gas volume.

Summary. Gas producing efficiency of raw and heated navy beans with or without antibiotic supplementation was evaluated and compared with that of casein diet. Raw navy beans produced 4 to 6 times as much gas as that produced by casein. On the other hand heated navy beans produced only one and one-half to 2 times more gas than that obtained on casein diet. Addition of antibiotics to the raw navy bean diet significantly reduced the flatus production in adapted rats but was without effect in unadapted rats fed raw or heated beans. The possible mechanism of increased gas production in the animals fed raw navy beans and the beneficial effect of supplementary antibiotics in reducing the gas production have been discussed.

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MSH Activity in Rat Pituitaries After Pinealectomy.*† (31986)

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The pineal gland of mammals produces melatonin, a substance which is the most active agent known to lighten amphibian melanocytes *in vitro*(1) and *in vivo*(2). It has been recently suggested that administration of melatonin may cause a decrease in the pituitary content of melanocyte-stimulating hormone (MSH) in albino rats(3).

Removal of the pineal gland, therefore, might be expected to result in an increased level of MSH in the pituitary. Accordingly, the following experiments were performed to determine the effect of pinealectomy upon rat pituitary MSH content.

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Materials and methods. Female Sprague-Dawley rats (Cheek-Jones Co., Houston) approximately 3 weeks old (50 g) and 9 weeks old (200 g) were pinealectomized by the method of Hoffman and Reiter(4). Rats serving as controls were subjected to an identical operation except that the pineal was left intact. Three days, 1 week, 4 weeks, or 8 weeks following operation the rats were decapitated and the whole pituitaries were removed, weighed, and stored at −20°C. At the time of assay, pituitaries from each group of 3-4 rats were pooled, homogenized in 0.1 N HCl-0.9% aqueous NaCl-0.1% bovine serum albumin, and kept on ice throughout the study. MSH activity was measured by an *in vivo* assay method involving injection of the test material into the dorsal lymph sac of

TABLE I. Effect of Pinealectomy on Pituitary MSH Content in 9-Week-Old Rats.

Time after operation	Operation	Pituitary MSH content (units/mg)	95% confidence limits	% Change
3 days	Sham operation	961	855–1067	
	Pinealectomy	2362	2055–2669	+146
	Sham operation	1151	1013–1289	
	Pinealectomy	2089	1880–2298	+ 81
1 wk	Sham operation	508	422– 594	
	Pinealectomy	840	706– 974	+ 65
	Sham operation	859	773– 945	
	Pinealectomy	1965	1690–2240	+129
4 "	Sham operation	949	816–1082	
	Pinealectomy	1459	1269–1649	+ 54
8 "	Sham operation	780	671– 889	
	Pinealectomy	741	660– 824	— 5

TABLE II. Effect of Pinealectomy on Pituitary MSH Content in 3-Week-Old Rats.

Time after operation	Operation	Pituitary MSH content (units/mg)	95% confidence limits	% Change
3 days	Sham operation	2327	2141–2513	
	Pinealectomy	4372	4022–4722	+88
	Sham operation	1426	1312–1540	
	Pinealectomy	2386	2243–2529	+67
	Sham operation	2337	2150–2524	
	Pinealectomy	2605	2240–2970	+11
1 wk	Sham operation	979	881–1077	
	Pinealectomy	917	853– 981	— 6
	Sham operation	1260	1109–1411	
	Pinealectomy	1289	1160–1418	+ 2
4 "	Sham operation	1074	924–1224	
	Pinealectomy	1059	921–1197	— 1
8 "	Sham operation	905	778–1032	
	Pinealectomy	944	784–1104	+ 4

hypophysectomized male frogs(5). The unit of MSH activity used in this report was defined by Shizume *et al*(6). ACTH activity was determined by a method described elsewhere (7,8).

Results. When 9-week-old rats were used, there was a significantly greater MSH content of pituitaries from pinealectomized rats as compared with the MSH content of pituitaries from sham operated control rats at 3 days, 1 week, and 4 weeks after operation (Table I). This effect was no longer evident 8 weeks after removal of the pineal gland from these rats.

In 3-week-old rats, a significant elevation of pituitary MSH content was also observed 3 days after pinealectomy in 2 out of 3 experiments (Table II). In contrast to the 9-week-old rats, however, no increase in MSH content was found 1 week or 4 weeks after pineal removal.

The cause of the variation in pituitary MSH levels among different experiments is not known. Preliminary evidence indicates that stress, constant illumination, and sodium loading may alter pituitary MSH content.

Since ACTH has intrinsic MSH-like activity, the observed effects might have been due to changes in ACTH activity. ACTH activity was measured but found not to be increased in the pituitaries of a group of 200 g rats which had been pinealectomized (3.01 mU/mg) as compared with a group which had been sham operated (3.53 mU/mg). The MSH content of the pituitaries from this pinealectomized group was 81% greater than that found in the pituitaries from the sham operated controls.

Discussion. Removal of the pineal gland from 9-week-old rats (Table I) resulted in an increase in pituitary MSH content which occurred within 3 days and appeared to persist at least 1 month. In 3-week-old rats a significant elevation of pituitary MSH levels was also observed in 2 out of 3 experiments 3 days after pinealectomy. However, this elevation disappeared within 1 week after removal of the pineal from these younger rats.

Melatonin has been shown to decrease pituitary MSH content(3). Conversely, MSH-release inhibiting factor (MIF) of hypothalamic origin increases levels of pituitary MSH(9,10). It might be reasonable to assume, therefore, that pinealectomy resulted in elevation of pituitary MSH content because of the absence of the stimulatory influence of melatonin or other pineal substances. Pinealectomy thus would be considered to have removed the mechanism which opposed the action of MIF.

The adjustment of pituitary levels of MSH toward control values may have involved, in addition to MIF, changes in circulating MSH(3) or a possible MSH-releasing factor (9,11). The feedback action of circulating MSH, however, may be mediated by MIF(3, 12). The results indicate that the compensatory adjustment following pinealectomy probably involved MIF and occurred more rapidly in the younger rats (Table II) than in the older ones (Table I).

Summary. Pinealectomy resulted in elevation of the MSH content of the pituitary glands of albino rats. This increased level of pituitary MSH persisted for several weeks in 9-week-old rats, but for only a few days in 3-week-old rats.

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Protection Against Lethality of Endotoxin with Isoproterenol: the Chick Embryo as an Experimental Model.* (31987)

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The 10-day chick embryo is very susceptible to endotoxin when administered by one of several routes. Protection against endotoxin is obtained with adrenocorticosteroids but not with heparin or phenoxybenzamine(1,2). Experimental and clinical evidence indicates that Isoproterenol is effective in endotoxin shock(3-7). In the present studies the chick embryo was used as a model to quantitate the lethality of intravenously administered endotoxin and protection of Isoproterenol.

Methods. White leghorn fertile eggs (Ghostley Pearl, Anoka, Minn.) received prior to incubation were used throughout. Ten-day eggs were candled, a large vein

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