TABLE II. Comparison of the Biological Activity of L-T_4 and L-T_3 by Subcutaneous Injection.

Hormone	Flighty No. of birds	Flighty Mean TSR (μg/100 g body wt)	Docile No. of birds	Docile Mean TSR (μg/100 g body wt)
L-T_4	15	.82	19	.49
L-T_3	15	.38	19	.23
Ratio L-T_4 to L-T_3		2.16		2.13

shown that L-T_3 was 2.6 times as active as L-T_4 in male rats(9), and 2.14 times as active as L-T_4 in dairy cattle(10). In raccoons, L-T_3 was 1.92 times as active in adult male and 1.06 times as active in juveniles(11). In the opossum, L-T_3 was found to be only 1.1 times as active at L-T_4(12).

No explanation can be offered for our observation concerning the increased biological activity of L-T_3 in this study compared to the previous reports in fowls.

Summary. Two strains of chickens developed by the Hy-Line Poultry Farms which were characterized by the terms as "docile" and "flighty" as to temperament were studied in regard to their estimated thyroxine secretion rate (TSR) during the summer (June). The "flighty" strain had a mean TSR of 0.82 μg L-T_4/100 g bw/day whereas the "docile" strain had a mean TSR of 0.58 μg L-T_4/100 g bw/day, a difference of 67.6%. In a second trial about one month later, during a period of elevated temperature 90°F (90-95°F), the TSR of the "flighty" birds was reduced to 0.58 μg/100 g bw, but the "docile" birds were unaffected. The percentage uptake of I^{131} by the "docile" birds was about 40% greater than the "flighty" birds indicating that the percentage uptake of I^{131} is not a good index of the functional activity of the thyroid gland. While previous studies indicated that L-T_3 and L-T_4 were approximately equal in biological activity in fowls, in this study, the two lines of birds showed L-T_3 to be slightly over twice as active as L-T_4.

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Received September 29, 1966. P.S.E.B.M., 1967, v124.

Effect of Hypophysectomy on Concentration of Intrinsic Factor In Rat Gastric Mucosa.* (31735)

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The purpose of this study was to observe the influence of hypophysectomy on the concentration of intrinsic factor (IF) in the gastric mucosa and, thereby, to throw light on two problems pertaining to the biology of IF. First, the cellular origin of IF remains in

* This investigation was supported in part by USPHS research grant AM-00131-13.

† Supported by a Medical Student Research Fellowship.

TABLE I. Effect of Hypophysectomy on Concentration of Intrinsic Factor in the Gastric Mucosa.

Treatment	No. of rats	Body wt (g) Initial	Body wt (g) Final	Vit. B_{12} uptake (mμg/g ileum/1½ hr)
Group I: *Ad libitum*-fed				
Hyp.	9	180 ± 3*	182 ± 5	1.71 ± .41
Nonhyp.	9	179 ± 2	307 ± 9	4.33 ± .33
			P	<.02†
Group II: Pair-fed				
Hyp.	12	181 ± 2	181 ± 4	2.89 ± .23
Nonhyp.	12	182 ± 2	221 ± 5	4.96 ± .39
			P	<.002

Hyp. = hypophysectomized.
* Mean ± standard error of mean.
† Mann-Whitney U test.

dispute. Second, the possible influence of hormones on production of IF has not been clearly defined. Hypophysectomy of the rat causes a profound involution and functional depression in the gastric chief cells with only mild alteration occurring in the other epithelial cell types of the stomach(1,2). Thus, the occurrence of a fall in the mucosal concentration of IF after hypophysectomy would indicate that chief cells secrete IF and implicate several hormones in the regulation of its production.

Methods. Young adult female Sprague-Dawley rats were fed a diet of Purina Laboratory Chow. At hypophysectomy they weighed 170-190 g. Each hypophysectomized rat was paired with a control from which the hypophysis was not excised. Two groups of experiments were carried out (Table I). In the experiments of Group I all rats were fed *ad libitum*; in Group II, each nonhypophysectomized rat was pair-fed against its operated mate. The period between hypophysectomy and termination of the experiments was 95 to 134 days for Group I and 77 to 94 days for Group II. The rats were decapitated while under sodium pentobarbital anesthesia following a 24-hour fast. Completeness of hypophysectomy was verified by microscopic examination of serial sections of the pituitary area. Rats retaining pituitary fragments were excluded.

After excision, the stomach was opened along the lesser curvature and rinsed with 0.9% saline. The mucosa was scraped from only the fundic area, excluding the mucosa along the lesser curvature. The mucosa was placed in cold bicarbonate-free Krebs-Henseleit buffer, its weight being determined by ascertaining the increase in weight of the buffer-containing flask upon addition of the mucosa. The mucosa was homogenized while maintained at low temperature.

IF concentration was determined by the method of Boass and Wilson(3) which is based on determination of the uptake of Co^{57}-labeled vitamin B_{12} by 1 mm thick rings of everted hamster ileum when incubated in the presence of IF. For each experiment, 3-4 male golden hamsters weighing 90-120 g were fasted 24 hours and ileal segments obtained after decapitation. The IF was provided by addition of rat gastric mucosal homogenate to the incubation medium. For each experiment 6 flasks containing gastric mucosa from a hypophysectomized rat, 6 containing mucosa from the nonhypophysectomized partner, and 6 containing no mucosa were incubated simultaneously. The 6 flasks containing no mucosa served as a control to show how much B_{12} was taken up by the ileal rings in the absence of IF. The volume of the incubation medium per flask was increased over that used by Boass and Wilson(4), the following proportions being utilized: 0.2 ml of 6% glucose, 0.8 ml of 2.1% $NaHCO_3$ previously gassed with 100% CO_2, 3.15 mμg of Co^{57}-B_{12} (1 mC/mg),‡ and 7 mg of mucosal homogenate in 6 ml of bicarbonate-free Krebs-Henseleit buffer for 10-15 ileal rings. Thus, the final concentrations of mucosa and of B_{12} were maintained constant in the incubation medium at 1 mg/ml and 0.45 mμg/ml, respectively. Incubation was carried out for 1½ hours at 37°C with the medium being gassed continuously by a mixture of 95% O_2 and 5% CO_2. After incubation the ileal rings were washed in 0.9% saline, weighed, and counted in a NaI well-type scintillation counter coupled to a single channel pulse height analyzer. The uptake of B_{12} as indicated by the radioactivity in the ileal tissue was considered to be an index of the IF present in the incubation medium.

‡ Vitamin Co^{57}-B_{12} was obtained from N. V. Philips-Duphar, Amsterdam, Holland.

For each experiment the mean uptake for the 6 flasks containing no mucosa was subtracted from the means for the 6 hypophysectomized rats and for the 6 nonhypophysectomized controls. Corrections for isotope decay, background counts, and counter efficiency were made and the results expressed as mμg of B_{12} uptake/g ileum/1½ hr. The mean uptake was then calculated for all hypophysectomized rats and for all nonhypophysectomized rats in each group of experiments. The significance of the differences between these means was determined by the Mann-Whitney U test(5).

Results. Considering together the 21 experiments of Groups I and II (Table I) the mean B_{12} uptake by the ileum in the absence of IF stimulation (no mucosal homogenate in the incubation medium) was 0.64 ± a standard error of the mean of 0.05 mμg/g ileum/1½ hr. Addition of one mg of mucosa from the nonhypophysectomized rats per ml of the incubation medium increased the uptake to 5.33 ± 0.27 mμg B_{12}. Preliminary tests had revealed that greater uptake could have been induced by increasing the concentration of the mucosa in the incubation medium.

Hypophysectomy decreased the IF concentration in the gastric mucosa (Table I). IF was 61% less concentrated in the hypophysectomized rats than in the nonhypophysectomized controls after *ad libitum* feeding (Group I) and 41% less concentrated after pair-feeding (Group II).

Discussion. In reviews of the literature pertaining to IF, Glass(6,7) cited evidence indicating that any one of the following epithelial cell types of the stomach may be the source of IF: mucous neck, parietal, argentaffine, and chief cells. Particular interest has been directed toward the mucous neck cell because both IF and the secretory product of these cells are considered to be mucoprotein in nature. A parietal cell origin is based chiefly on selective binding of B_{12} to parietal cells in man. However, the following evidence points more convincingly to the chief cell as being involved in the elaboration of IF. The area of the gastric mucosa, which normally contains the highest concentration of chief cells, atrophies during pernicious anemia (PA) and chief cells disappear. Secretion of both pepsin and IF is elicited by histamine and vagal stimulation. At different levels in the fundic mucosa of the rat the concentrations of pepsin and IF vary in parallel(4). Finally, as shown by radioautography performed on microscopic sections of gastric mucosa from rats, B_{12} binds selectively to chief cells, the binding material, like IF, being thermolabile and the binding being blocked by IF antibody obtained from the serum of PA patients.

Our observations tend to support the chief cell hypothesis. As observed in the rat by light(1,2) and electron microscopy(8), the surface, mucous neck, and argentaffine cells exhibit little or no structural change following hypophysectomy. Indeed, the amount of mucoprotein contained in mucous neck cells may even be increased. Parietal cells become slightly smaller, and exhibit minor structural changes when observed with the light microscope(1,2) but these have not yet been confirmed *in toto* with the electron microscope (8). Alteration in secretion of hydrochloric acid is minimal(9) and concentration of parietal cells in the rat gastric mucosa remains unchanged(10). Robert *et al*(11) reported a reduction in secretion of mucus after hypophysectomy but the cellular source of this deficiency was not identified.

In contrast to the minimal alteration induced by hypophysectomy in most epithelial cell types of the stomach, chief cells are affected profoundly. A reduction of 85% in total pepsin and of 64% in concentration of pepsin in the gastric juice is associated with a marked decrease in size of chief cells and their nuclei, in number of mitochondria and secretory granules, intensity of cytoplasmic basophilia(1), amount of rough-surfaced endoplasmic reticulum and in size of the Golgi apparatus(8). Thus, the depression in concentration of IF in the gastric mucosa induced by pituitary ablation appears to be accounted for by atrophy of the chief cells.

Little information is available concerning the possibility that IF production is controlled by hormones. Most pertinent studies have involved therapy of patients with PA. Prior to Glass' review(6), a favorable hematopoietic

response was obtained by treatment with corticotropin or corticosteroids. However, in these studies enhancement of IF secretion was not differentiated from general improvement in intestinal absorption as being the reason for the clinical effect observed. More recently, accelerated secretion of IF has been demonstrated in some cases of PA following corticosteroid therapy(12,13,14). Pernicious anemia occurs occasionally in conjunction with Addison's disease and in some hypothyroid patients(6). The absorption of B_{12} is deficient in human hypothyroidism(15). The demonstration that pituitary hormones affect the mucosal content of IF raises the possibility that a hormonal derangement may be involved in the etiology of PA.

The nature of the hormonal pattern required for maintenance of a normal concentration of IF in the gastric mucosa of the rat can only be conjectured at this time. Treatment with a combination of somatotropin, corticosterone and thyroxine restores the reduced weight and involuted acinar cells of pancreas and parotid gland in hypophysectomized rats to approximately normal. However, only incomplete restoration is effected in gastric chief cells(16) although full recovery follows implantation of the hypophysis(17). Since adrenalectomy causes some involution of chief cells(18) and reduces secretion of pepsin(19), and since combined adrenalectomy, thyroidectomy and gonadectomy elicits changes comparable in severity with those which follow hypophysectomy(19), one may infer that the hormones produced by one or more of these glands play a significant role in promoting the synthesis of IF.

Summary. Hypophysectomy caused a 41% decrease in the concentration of IF in the fundic mucosa of the rat even when the food intake of control rats was limited to that of the hypophysectomized rats. Since the chief cell undergoes more profound involution and functional depression than any other cell type in the fundic gland, these observations point to the chief cell as being the source of IF production in the rat. Also, the normal elaboration of IF appears to be under hormonal control.

We thank Dr. Claire J. Shellabarger, Director, Radioisotope Research Laboratory, Univ. of Michigan, for consultation regarding technical aspects of the work, and the staff of the Statistical Research Laboratory for advice on statistical treatment of the data.

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