The relationship of intraglandular colloid production to hormone synthesis[1]

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Summary. The production of intraglandular colloid, brought about by the cyclic breakdown of intermediate lobe cells, is related to the synthesis of hormones in the hypophysis. By analyzing the 'shift' of the amino acid, cystine, during various phases of the gland's cycle it is apparent that hormones, as well as other protein determinants of immunological importance, are transported by way of the intraglandular cleft to the venous circulation and to the cerebrospinal fluid.

During the active phase of hypophyseal intraglandular colloid production[2], i.e., the breakdown of intermediate lobe cells resulting in the formation of colloid, the synthesis of hormone ceases. This phenomenon was recognized during our analysis of glandular material for the amino acid, cystine, as it was necessary to investigate a specific biological entity common to proteins and hormones.

We chose to analyze for this amino acid, because it is responsible for disulfide bonding of biological molecules, in particular immunoglobulins, and performs a functional role in maintaining the stability and establishing the configuration of certain hormones[3].

Encapsulated glands from young beef-type steers were removed within 20 min of sacrifice, quick frozen in liquid nitrogen and then stored at −20 °C. Allowed to stabilize at ambient temperature, the glands were sliced mid-sagitally and immersed in normal saline. Intraglandular colloid, when present, was removed. The glands were then separated into 2 groups: 1 group showing colloid formation, the other, the glands devoid of colloid. The glands were grossly sectioned and portions of anterior, intermediate and posterior lobes taken for further analysis. Individual pools were homogenized in a teflon/glass tissue grinder, washed in equal amounts of saline, and centrifuged at 3000 × g for 20 min to separate the cellular material from soluble proteins and/or peptides. Each pool was passed through a Beckman model 24 spectrophotometer and the optical density recorded at 280 nm. With an arbitrary extinction coefficient of 1.35, the material was diluted (or concentrated) to represent 5 mg/ml. This material was again scanned spectrophotometrically (340 nm - 300 nm) to ensure that no hemoglobin (hence, residual blood) was present in the intraglandular colloid. Amino acid analysis (Beckman model 20) of the soluble portion of glands which did not contain intraglandular colloid (i.e., the intermediate lobe was intact and in contact with the anterior lobe) recorded the levels of cystine shown in figure 1 (A,B,C). These levels are representative only of the pool of glands used in this study and should not be considered 'standard'. However, when the breakdown of marginal cells of the intermediate lobe forms colloid in the intraglandular cleft[2] there are barely discernible levels of cystine in the soluble portion of the lobes (figure 1, D–F) although the colloid in each case (figure 1, G,H) exhibits elevated levels.

It must be understood that intraglandular colloid is found in a range of consistencies from a solid mass through a gelatinous to a free flowing liquid. In this study we arbitrarily divided it into 2 pools based on viscosity: 1 containing material from a solid to a viscous gel (figure 1, G), the other a soft gel to a fluid (figure 1, H).

Growth hormone, synthesized in the anterior lobe of the pituitary, is a multichain molecule held together by disulfide bonds, the number and position depending on the species. Since our study shows no detectable cystine in glands undergoing the breakdown of intermediate lobe cells, and elevated levels in the colloid, we conclude that: 1. the anterior lobe does not synthesize growth hormone during this phase, and 2. segments of the molecule containing the disulfide bridges are transported, by mechanisms not yet fully understood, into the intraglandular cleft to be absorbed and distributed by the colloid.

Although investigators seem to disagree on the possible species crossreactivity of certain hormones, evidence is very strong that several hormones have been shown, on analysis, to overlap sequences of amino acids[3] which may, or may not, be instrumental in the overall function of the particular hormone. These sequences could, conceivably, originate (or terminate) in serum proteins (i.e. immunoglobulins), as there is no evidence in the literature which contradicts this premise.

To localize the immunoglobulin presence in the pituitary, the samples used for amino acid analyses were electropho-

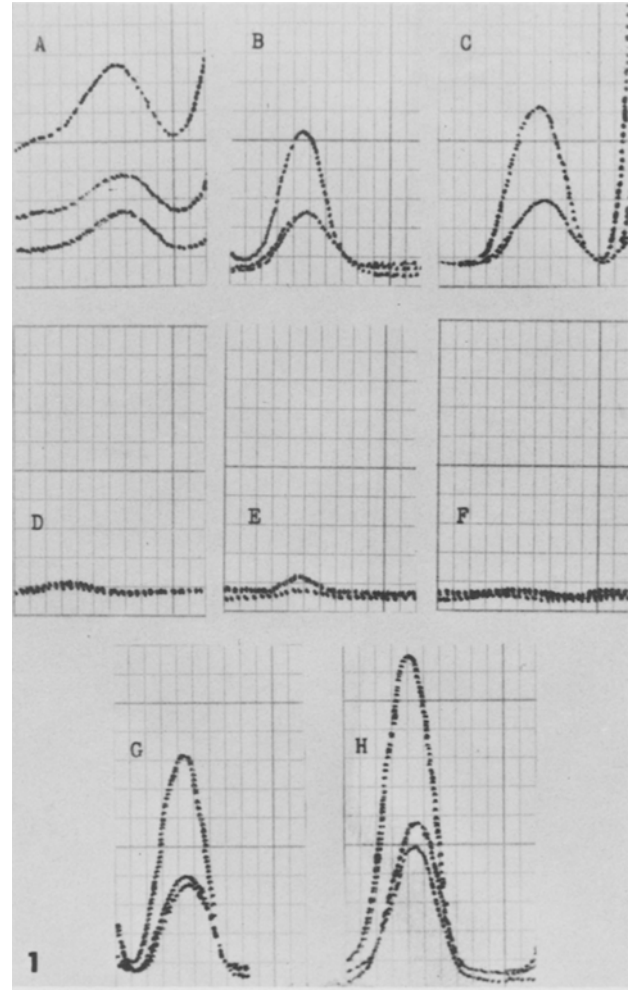

Fig. 1. The amino acid cystine isolated from recordings of pituitary materials after acid hydrolysis. *A*, anterior; *B*, intermediate; *C*, posterior lobes of glands which contain no discernable colloid; *D*, anterior; *E*, intermediate; *F*, posterior lobes of glands from which colloid has been harvested; *G*, solid-viscous gel; *H*, soft gel-fluid phases of intraglandullar colloid.

resed for 20 min in a Gelman electrophoresis chamber in 1% Noble agar equilibrated to 0.01 M veronal buffer, pH 8.4, which was also the electrode buffer. Troughs were filled with rabbit antiwhole bovine serum and the resulting patterns stained with amido black 10 B.

The sequence in figure 2 shows the posterior lobe (figure 2, A) devoid of serum-like proteins except for an arc in the albumin region regardless of the presence (bottom) or absence (top) of colloid in the gland. The anterior lobe (figure 2, B) shows a complement of serum proteins, including immunoglobulins, in glands without colloid (top), but again, only the arc in the albumin region when colloid is present (bottom). Intermediate lobe (figure 2, C) shows traces of protein in the 'gamma' regions as well as a strong albumin-like arc in glands with colloid (top), whereas material from glands without colloid (bottom) displays a pattern depicting a range of proteins similar to bovine serum[4]. The lower photo (figure 2, D) shows the pattern of colloid in the fluid-gel state containing a range of proteins from fast gamma to alpha. Of interest is the arc in the slow gamma/beta region where one might expect to find serum immunoglobulins A and/or M.

If then, pituitary materials, specifically intraglandular colloid, show immunoreactivity to antisera to whole bovine serum, how do they relate to other biological material when tested against specific protein antisera? Figure 3 shows an immunoreactive determinant shared by growth hormone (GH), follicle stimulating hormone (FSH), cerebrospinal fluid (CSF), and high and low molecular weight fractions of intraglandular colloid when diffused toward antisera to bovine serum IgG, and another toward anti bovine serum albumin (BSA).

Although all the samples herein tested are of one species (bovine) there is little doubt that species cross-reactivity is the result of these same determinants, i.e., short chains of amino acids chemically linked in the proper sequence.

Accessibility of intraglandular colloid to the CSF by way of the intraglandular lumen and the subarachnoid space[5], to the systemic circulation via the cavernous sinuses[5], and to the hypothalamus via the pituitary stalk[3,7,8], elevates its role from a waste product to a transport mechanism in the regulation of hormone supply, and may even play a func-

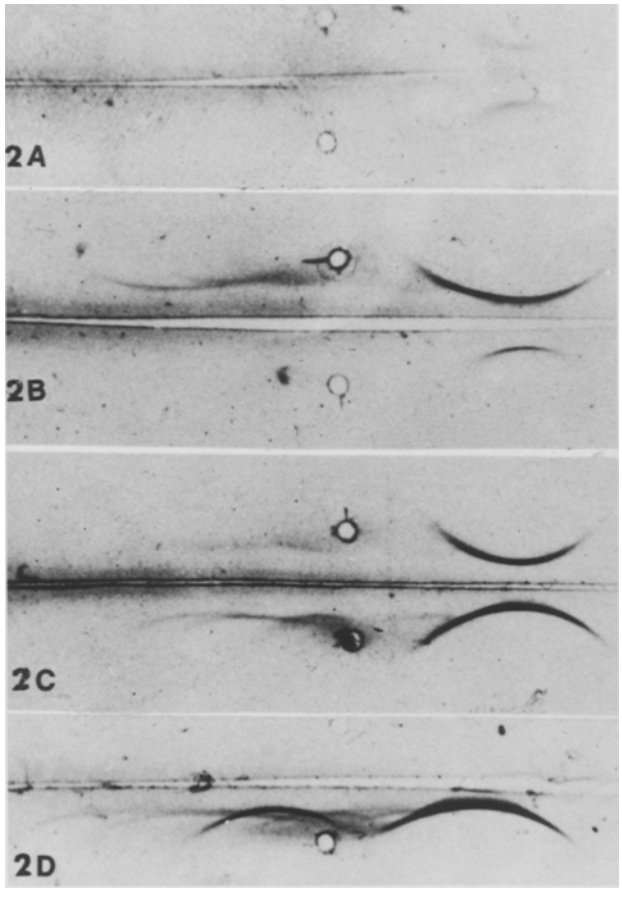

Fig. 2. Immunoelectrophoresis of pituitary materials. *A*, posterior lobe, from gland without colloid (top) and with colloid (bottom); *B*, anterior lobe, without colloid (top), with colloid (bottom); *C*, intermediate lobe, with colloid (top), without colloid (bottom); *D*, fluid phase intraglandular colloid (bottom). All troughs contain rabbit anti whole bovine serum.

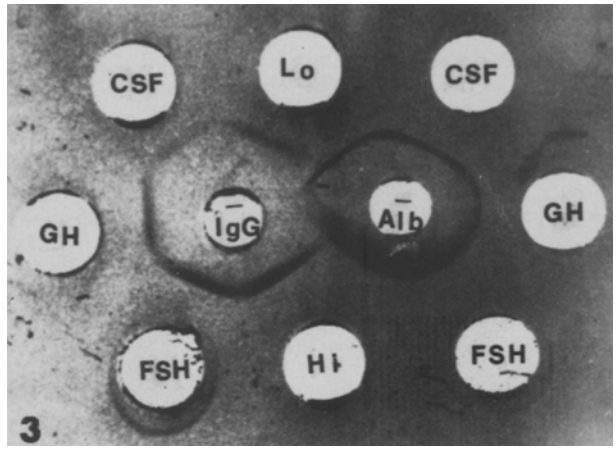

Fig. 3. Double diffusion in agar showing similar immunoreactive determinants in pituitary hormones, cerebrospinal fluid and colloid fractions against antisera to bovine serum proteins. GH, growth hormone; FSH, follicle stimulating hormone, (gifts of NIAMD through the National Pituitary Agency). Anti IgG and anti Alb purchased from Miles Laboratories. CSF, bovine cerebrospinal fluid (a gift of OVC clinics); Hi, high molecular weight fraction; Lo, low molecular weight fraction (of intraglandular colloid, prepared in our laboratory).

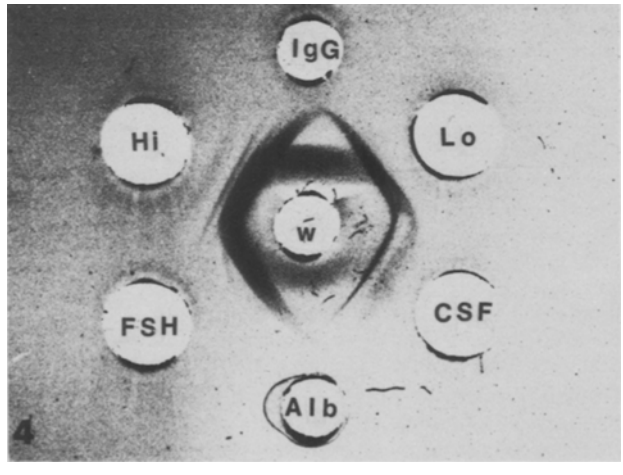

Fig. 4. Double diffusion in agar showing the common determinant in colloid fractions, follicle stimulating hormone (FSH) and cerebrospinal fluid (CSF), which does not identify to serum IgG nor to albumin (Alb). Anti whole bovine serum (anti w) from Miles-Yeda; serum antigens IgG and albumin, from Miles Laboratories.

tional role in the control of immunoglobulin precursors to CSF and to the circulatory system.

The obvious conclusion would be that hormone preparations are contaminated with serum IgG and albumin. Different hormones are expected to diffuse at different rates. Although this is true for reactions between whole molecules against specific countering antisera, we cannot ignore the fact that immunoreactions of similar determinants, originating within non-identical antigens, would show immune identity. This is clearly indicated in figure 4. Serum IgG identifies with determinants in the intraglandular colloid fractions and CSF as expected. However, when these fractions, and FSH are diffused against antiserum to whole bovine serum the major precipitate does not identify with either serum IgG or albumin.

These facts strongly support our contention that the production of intraglandular colloid, brought about by the cyclic breakdown of intermediate lobe cells, is related to the synthesis of hormones in the hypophysis. Furthermore, the accessability of this holocrine secretion to the venous circulation and to the cerebrospinal fluid suggests its implication in the transport of immunoreactive determinants.

1 This study was supported by a grant from the National Research Council of Canada.
2 W.H. Boyd, Archs Histol. Jap. *34*, 1 (1972).
3 D.T. Krieger and A.S. Liotta, Science *205*, 366 (1979).
4 J.E. Butler, Dairy Sci. *52*, 1895 (1969).
5 W.H. Boyd, Anat. Anz. *133*, 461 (1973).
6 W.H. Boyd, Anat. Anz. *133*, 454 (1973).
7 R.M. Bergland and R.B. Page, Endocrinology *102*, 1325 (1978).
8 R.M. Bergland and R.B. Page, Science *204*, 18 (1979).

Effects of thyrotrophic-releasing hormone (TRH) on thermoregulation in the rat[1]

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Summary. At ambient temperatures (T_a) of both 8 and 22 °C, intraventricular administration of TRH (10–80 µg) produced a dose-dependent hypothermia in rats. The hypothermia was due to both decreased metabolic heat production and cutaneous vasodilatation. In contrast, at 30 °C T_a, TRH increased metabolic heat production (due to behavioral excitation) and led to hyperthermia.

Thyrotrophic-releasing hormone (TRH, pGlu-His-Pro NH_2) is found in, and is active on, neural processes throughout the brain[2–5]. In addition to its thyrotrophic action on the release of thyroid stimulating hormone (TSH), it has been shown that TRH in animals can reverse the CNS depression induced by barbiturates and alcohol[6,7]. Recently, Metcalf, Myers and Rice reported that intracranial injections of TRH produced hypothermia in cats but hyperthermia in rabbits[8–11]. In the present study, the effects of intraventricular injections of TRH on thermoregulatory outputs (including metabolic, respiratory and vasomotor activities as well as body temperatures) were assessed in the rat at various ambient temperatures (T_a).

Materials and methods. Adult male Sprague-Dawley rats weighing 250–300 g were used in all experiments. Measurements were obtained from conscious animals which were trained to sit quietly under minimal restraint in rat stocks. Between experiments the animals were housed individually in wire-mesh cages in a room of 25 ± 1.0 °C with a 12:12 h light-dark cycle. The animals were given free access to tap water and granular chicken feed. For intraventricular injection, ventricular cannulae were chronically implanted in the animals under anesthesia (sodium pentobarbital, 6 mg/100 g, i.p.). Implanatation of ventricular cannulae were carried out according to the DeGroot coordinates: AP, 7.0; Lat., 1.0; and Hor., 0.1 mm[12]. A 27-gauge Hamilton syringe needle was connected via PE-10 tubing to a 50-µl Hamilton syringe. During the surgery the correct positioning of each guide tube was verified by the rapid flow of saline or drug solutions into the lateral cerebral ventricle under gravity. At least 2 weeks were allowed for the animals to recover from the operation. All drug solutions were prepared in pyrogen-free glassware which was baked at 180 °C for 5 h before use. A 5-µl aliquot containing 10–80 µg TRH (Sigma) was administered into the lateral cerebral ventricle through a guide tube. Metabolic rate (M), respiratory evaporative heat loss (E_{res}) and vasomotor activities were measured in a small calorimeter[13–15]. M was calculated from the animal's oxygen consumption and expressed as W/kg b.wt. E_{res} was calculated by measuring the increase in water vapor content in the expired air. Evaporative heat loss expressed as W was calculated from evaporative water loss[13–15]. Rectal (T_r), foot skin (T_f) and tail skin (T_t) temperatures were measured using copper-constantan thermocouples. Rectal temperature was measured with a copper-constantan thermocouple enclosed in PE-200 tubing, sealed at one end, inserted 60 mm into the rectum. All measurements were taken once per 1 min throughout the experiments, each variable being measured as a direct current potential on a Hewlett-Packard digital voltmeter (DVM 3465) interfaced to an on-line CPU 9825 computer. Every min all temperatures, M and E_{res} were calculated instantaneously by the computer and relayed immediately back to the laboratory where they

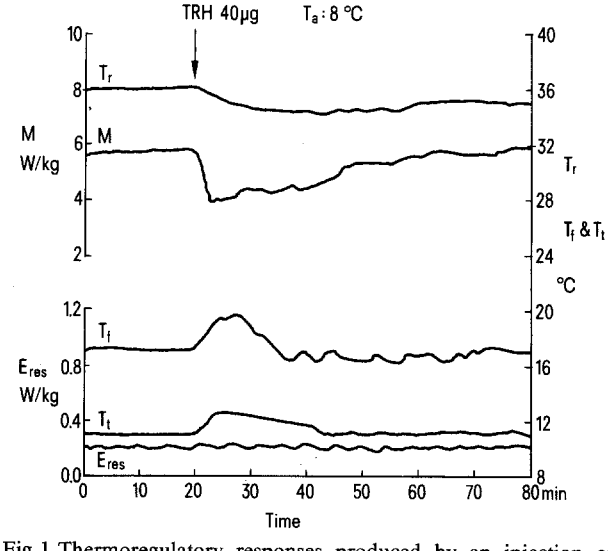

Fig. 1. Thermoregulatory responses produced by an injection of TRH into the lateral cerebral ventricle of a conscious rat at an ambient temperature of 8 °C.