SPECIAL ARTICLES

BIOLOGICAL EFFECTS OF HOMOLOGOUS THYMUS IMPLANTS IN SUCCESSIVE GENERATIONS OF RATS[1]

IN a previous communication, attention was called to the accruing acceleration in the rate of growth and development when successive generations of rats were given daily intraperitoneal injections of 1 cc of potent thymus extract (Hanson). It seemed likely that, if these phenomena were due to a substance inherent to the thymus gland and present in the extract, frequent implantations of homologous thymus implants in successive generations of rats would produce similar results.

Accordingly, 3 pairs of albino rats of the Wistar strain were given weekly implants of whole thymus glands, each animal receiving a total of 12 implants. The glands were obtained from stock rats between the ages of 20 to 50 days. Starting with the third generation, the implants were made at 5-day intervals. These implants were placed subcutaneously in different sites on the back and neck of the recipient. Sloughing of about one third of the implants was noted. The remainder were absorbed and grossly disappeared in about 2 weeks.

In transplanting the thymus gland, the recipient was first fastened by a specially constructed holder, an area of the back prepared by cutting the hair and infiltrating a small area of the skin with a few drops of 1 per cent. procain solution. An incision was made through this area, a subcutaneous pocket prepared and the surface of the underlying muscles abraded. A mattress suture was inserted and the first knot loosely tied. The donor was stunned, its thymus gland immediately removed and transferred to the prepared site in the recipient. Not more than thirty seconds elapsed between the time the donor was sacrificed and the final tying of the mattress suture in the skin of the recipient.

To date four successive generations of animals implanted with thymus glands have been observed. An analysis of the biological data of each of these generations reveals certain significant facts. In the first generation (F_0), no effect was apparent. The litters were large, the offspring at birth strong and of good size. In the second generation (F_1), there was noted a slight increase in the rate of growth, as revealed by body weight, especially between the tenth to the seventeenth days of life, reaching a maximum excess of 9 per cent. at the age of 40 days. In the third generation, the increase in growth was greater than in the second, again reaching a maximum excess of 12 per cent. at the age of 40 days. Three litters have been born in the fourth generation. As in preceding generations, acceleration in growth was noted, reaching a maximum excess of 16 per cent. at the age of 40 days.

The animals in the third and fourth generations were vigorous, and active. At the age of 12 to 14 days, they scampered about their cages in a manner like that of the control animals at the age of 20 days. One litter of the fourth generation was successfully weaned at the age of 12 days and given their first implants at the age of 15 days.

A study of the somatic changes revealed findings more striking than the increase in the rate of growth. Reference to Table 1 shows that in the second generation there was only a slight acceleration in the opening of the ears, the eruption of the teeth, the appearance of hair, the opening of the eyes, the descent of the testes and in the opening of the vagina. In the third generation, these somatic changes were further accelerated, while the most marked acceleration was observed in the fourth generation. Thus in the latter generation, the average time for eruption of the teeth, appearance of hair, opening of the eyes, descent of the testes and opening of the vagina was 5, 9, 7, 24.3 and 36 days, respectively (Table 1).

TABLE 1

COMPARISON OF SOMATIC DEVELOPMENT IN SUCCESSIVE GENERATIONS OF THYMUS IMPLANT STRAIN OF RATS WITH DEVELOPMENT OF CONTROL ANIMALS

	Ears open	Teeth erupted	Hair appeared	Eyes open	Testes descended	Vagina open
Controls	2–3 (2.9)	7–10 (8.5)	11–14 (12.5)	13–17 (15.2)	24–35 (30.2)	39–50 (44.3)
F_1	2–4 (2.9)	7–9 (8.1)	12–16 (13)	13–16 (14.7)	25–33 (28.2)	39–56 (44.6)
F_2	2–3 (2.5)	6–9 (7.1)	11–13 (12.1)	9–16 (13.1)	19–30 (24.5)	37–52 (40.7)
F_3	2–3 (2.2)	4–9 (6.8)	10–13 (11.3)	9–14 (11.8)	20–28 (24.6)	36–42 (39)
F_4	1–3 (2)	3–7 (5)	8–10 (9)	5–9 (7)	18–28 (24.3)	32–42 (36)

The experimental data here presented indicate that frequent homologous thymus implants into albino rats result in an accruing acceleration in the rate of growth and of development of the young. Although this acceleration is not so marked quantitatively as that resulting from the daily intraperitoneal injections (1 cc) of thymus extract, yet qualitatively it is in every respect identical in nature, the difference in results probably being due to the quantitative difference in the

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active principle concerned. These experiments with homologous implants afford important confirmation of our previously reported evidence as to the biological effect of thymus extract.

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THE SECRETION OF AN ANTIDIURETIC HYPOPHYSEAL HORMONE IN RESPONSE TO THE NEED FOR RENAL WATER CONSERVATION

EVIDENCE that the posterior lobe of the pituitary gland secretes a hormone concerned with the normal regulation of mammalian water metabolism is still incomplete. The classification of the posterior hypophysis as a gland of internal secretion is the result of two types of experimentation: first, the chemical isolation from the hypophysis of a substance possessing marked anti-diuretic activity in normal, unanesthetized hydrated animals, and, second, the occurrence of polyuria and polydypsia following degeneration or removal of the posterior pituitary and their relief by administration of pituitary extracts. From such evidence it has been deduced that the hypophysis secretes a hormone which enables the kidney to reabsorb water against an osmotic pressure in the renal tubule higher than that of the blood. No one, however, has demonstrated the presence in any body fluid of an anti-diuretic substance secreted by the posterior hypophysis in response to a physiological need.

The regulation of water retention and excretion in the mammalian organism is so well adjusted that the degree of body hydration is maintained within narrow limits. If renal water reabsorption is under hormonal control, an exceedingly labile relationship should exist between the secreting gland and the end organ to permit such accurate function. For such a labile system, two requirements are necessary: (1) the degree of hypophyseal activity must be readily adjusted to the body requirements for renal water reabsorption, and (2) the hormone must be easily inhibited or destroyed to permit diuresis following hydration. The postulate of a labile system does not seem to be fulfilled when one considers that the polyuria of diabetes insipidus can be relieved for many hours by the administration of a single unit of pitressin. That this is many times the effective dose, however, has been shown by Theobald,[1] who observed antidiuresis in one individual after the administration of 0.0005 units. That pitressin is readily destroyed by the body has been clearly demonstrated by Heller and Urban,[2] who proved its rapid inactivation by blood and tissues. They also observed that a portion of the injected pitressin is excreted in the urine. It would appear, therefore, that the kidney is extremely sensitive to minute amounts of this pituitary extract and the body is capable of rapidly eliminating amounts exceeding its physiological requirements.

The above facts suggest that the secretion of an antidiuretic substance is capable of effecting an accurate and sensitive control of renal water reabsorption. The demonstration that such a control actually exists and that it represents a true hormonal regulation of water reabsorption would be complete if it could be shown that the anti-diuretic substance is secreted in amounts varying with the need for water conservation. This necessitates the detection of this substance in body fluids and a method of quantitative estimation sufficiently sensitive to measure physiological variations in amounts.

The logical body fluid to examine for the presence of an anti-diuretic hormone would be the blood, were it not for the fact that extremely low concentrations are physiologically effective, and such minute amounts are readily inactivated. The observation that injected pituitrin passes through the glomerulus and is excreted in the urine, a medium in which the hormone is presumably more stable, offers another body fluid in which an anti-diuretic substance might be demonstrated. Heller and Urban failed to detect anti-diuretic activity in normal rat urine. If one increases the need for water reabsorption by dehydration, presumably this should result in greater hypophyseal activity and the urinary excretion of the anti-diuretic hormone in increasing amounts. The experiments to be described are based upon the above postulates.

Rats, dogs, monkeys and man were used as experimental subjects. Dehydration was accomplished either by the oral administration of 5 per cent. NaCl solution or by water deprivation over periods varying up to 72 hours. Urine was collected in vessels containing sufficient 1 per cent. acetic acid to make the final sample weakly acid. The urine was dialyzed through a Cellophane membrane to remove salts and urea which would interfere with the anti-diuretic assay. It was then concentrated *in vacuo* to a small volume and the antidiuretic activity determined by the rat method of Burn as described by Heller and Urban.[2] This method is based upon the rate of renal excretion of water in rats that have been hydrated to the extent of 5 per cent. of their body weight. Each sample was assayed collectively on four rats, the time for the excretion of 50 per cent. of the fluid administered being taken as the index of antidiuresis. A difference of five milliunits of pitressin can be detected by this method. The procedure of dialysis was controlled with known solutions of pituitrin, which showed no demonstrable loss

[1] G. W. Theobald, *Clinical Science*, 1: 225–239, 1934.
[2] H. Heller and F. F. Urban, *Jour. Physiol.*, 85: 502–518, 1935.