TABLE V. Levels of Epinephrine-Stimulated Lipase in Tissues of Rats Before and 30 Minutes After Heparin Injection.

	μeq fatty acid/g of wet tissue or ml plasma/hr			
	Before heparin injection		30 min after heparin injection	
Tissues	No epinephrine	Δ Increase due to epinephrine	No epinephrine	Δ Increase due to epinephrine
Heart	31.1 ± 3.8	14.8 ± 3.3	33.0 ± 9.6	.07 ± 2.0
Adipose tissue	26.1 ± 4.3	19.2 ± 3.3	23.0 ± 7.6	1.1 ± 1.9
Kidney	37.9 ± 9.0	30.4 ± 7.0	42.0 ± 4.6	2.8 ± 2.4
Liver	30.6 ±10.3	23.4 ± 8.3	35.0 ± 4.3	4.4 ± 2.7
Lung	24.3 ± 4.3	15.3 ± 9.3	38.0 ± 9.0	.1 ± 1.4
Plasma	2.3 ± 1.9	2.7 ± .9	6.6 ± 1.3	—.5 ± .015

Rats were sacrificed before and after injection of heparin. Tissues were removed, divided into 2 halves, weighed and placed in a solution containing 2 ml of 0.06 M phosphate buffer at pH 7.1 and 1 ml of 10% bovine serum albumin adjusted to pH 7.1 and incubated for 3 hr at 37°C. One half of the tissue was stimulated by addition of 30 μg epinephrine (final concentration 10 μg/ml) to the incubation mixture and was incubated 10 min longer. The other half of the tissue served as a control. Tissues were then homogenized in a suitable volume of 0.06 M of phosphate buffer at pH 7.1 to give a concentration of 50 mg/ml of wet tissue in the final incubation mixture described for assay of the β-monoglyceridase(6). The substrate used in these experiments was Ediol (4 mg coconut oil/ml of incubation mixture). Incubation period after addition of the substrate was 20 min. Free fatty acids were assayed as described in text(4). Data presented above are mean values of 3 experiments, and ± stands for variation from the mean.

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DNA in Human Pituitary Glands.* (32046)

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The cellular mechanism by which pituitaries become substantially different in weight is not fully known. There appear to be several possible mechanisms; one is hyperplasia, another is hypertrophy and still another may be a shift of one cell type to another. The ex-

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ploration of this question is related to, but not identical with, the question of how pituitary cells respond to stimuli calling for increases or decreases in pituitary hormone production or release. The recent burgeoning of the literature on hypothalamic control of the pituitary has made these questions more immediate: advocates of a more passive role for the hypophysis would expect that functional changes could and would occur independent of structural changes in the pituitary *per se* while those who continue to acclaim the hypophysis in its role as symphony conductor would opt for structural realignments consonant with the functional changes.

DNA determinations in human pituitaries obtained at post mortem are the basis for this report. Zamenhof and others(1,2) have used changes in total DNA and weight of brain tissue as a means of determining hyperplasia. Clifton(3) has measured DNA concentrations and changes in pituitary weights to assess hypertrophy *vs* hyperplasia in tumor bearing mice. Here, for the human pituitary, we determine total DNA, DNA concentration/g weight, and the range of these values in the normal adult male.

Materials and methods. Human male pituitaries were obtained at autopsy, weighed, frozen and a portion used for DNA determinations. Autopsy records and clinical histories of all donor patients were reviewed and 3 weight groups were compiled from patients in whom there was no record of endocrine abnormality or hormonal therapy.

A modification of the Rosen(4) procedure for DNA extraction and determination was followed. This method has been lately confirmed in its value by Skidmore and Duggan (5) inasmuch as the hot PCA treatment eliminates the error due to the hyperchromicity of DNA.

The frozen pituitaries were thawed, separated into anterior and posterior lobes, weighed, and homogenized. The homogenized gland tissue was allowed to stand in 1 M cold (4°C) perchloric acid (PCA) overnight to extract the RNA and acid soluble materials. After centrifugation in a Sorvall superspeed refrigerated centrifuge at 7000 RPM for 10 minutes with S-34 rotor, the pellet, prevalently containing DNA and protein, was resuspended and heated at 80°C for 20 minutes in 1 M PCA (final concentration). Centrifugation at 7000 RPM for 10 minutes was again performed and the amount of DNA contained in the supernate was determined on the basis of its absorption at 263 mμ and the absorption of known amounts of commercial DNA treated the same way as the samples. Standardization with the commercial DNA (calf thymus DNA supplied by Sigma Chemical Co.) was repeated in each experiment to control experimental variation closely.

The flow sheet presented as Fig. 1 summarizes this procedure.

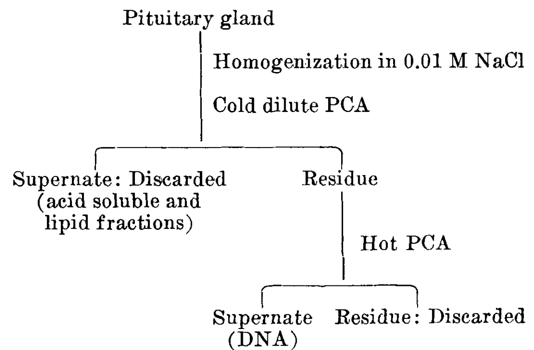

FIG. 1. Flow chart for tissue preparation.

The indole method of Ceriotti(6) and Stumpf's modification(7) of Dische's cysteine reaction(8) for determination of DNA were also used to determine the quantity of DNA in the final supernate. These results confirm the values obtained utilizing absorption at 263 mμ.

The Stumpf modification for DNA determination was used also to check loss of DNA at each step of the extraction. No significant loss was found.

Results. The mean weight of total anterior pituitary DNA in the small, average, and large pituitaries was found to be .742, 1.089, and 1.236 mg respectively. The mean DNA concentration was approximately equal in these groups with a range of 2.075-2.281 mg/g of pituitary tissue.

The DNA concentration of 34 posterior pituitaries was determined. A mean of .72 mg/g of pituitary tissue was found with a

TABLE I. Determination of Total DNA and DNA Concentration in Human Anterior Pituitary Glands of Differing Weight and Endocrine States.

Pituitary group	Mean pituitary wt in g (range of values)	No. of glands	Mean total DNA in mg (standard deviation)	Mean DNA concentration in mg/g (standard deviation)
Small	.327 (.303–.349)	12	.742 (±.069)	2.281 (±.204)
Average	.465 (.453–.485)	13	1.089 (±.258)	2.331 (±.552)
Large	.597 (.546–.668)	7	1.236 (±.171)	2.075 (±.274)

range of .48 to 1.16 mg/g. Thus, the DNA concentration of posterior pituitary is approximately 30% of anterior pituitary. Because the results on posterior lobes were obtained from pooled samples owing to their low weight, direct comparison between the anterior and posterior pituitary in a given case cannot be made.

Discussion. The similar DNA concentration in the 3 weight groups indicates a constant amount of DNA per cell. Thus the possibility of cellular hypertrophy in glands of different weights is ruled out. Indeed, since the total amount of DNA in these glands is correlated to their weight, it seems more reasonable to explain the variation in size with variation in number of cells.

The results that we obtained in glands of varying weight are compatible only with hyperplasia as the basis for increased gland weight in the endocrinologically normal human male. This empirical finding does not, however, indicate how the increase in cell population is produced. Hyperplasia seems to imply multiplication of cells, but from the literature it is known that in normal adult glands mitotic figures are not encountered. The question then arises when and how the mitotic division would take place. Whether there is another way of explaining the increase in number of cells per gland requires further study.

Even though the biochemical evidence of hyperplasia in the normal male with a large gland is incontrovertible we have no evidence that this is related to altered function. It is generally assumed that alterations in size may be associated with alterations in function but we have excluded such functional alterations from consideration in the weight groupings.

Summary. DNA measurements in normal human anterior pituitary glands reveal a constant concentration per unit weight and increased DNA in larger glands. This suggests that new cell formation (hyperplasia) is responsible for increased gland size.

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