

the administration of phenolsulphonephthalein the glomerular capsule can be seen to be distended with pink fluid indicating that it is excreted through the glomerulus in an alkaline urine. Rowntree and Geraghty^{1, 2} have previously shown that phenolsulphonephthalein is excreted through the tubules of the kidney but made no observations upon the excretion through the glomeruli. Under direct observation phenolsulphonephthalein can also be seen in the renal tubules, usually as a red dye indicating a deep alkalinity. The tissues immediately surrounding the renal capillaries are in some animals stained a diffuse red by the phenolsulphonephthalein and in some a light yellow, indicating that the tissues are sometimes alkaline and sometimes acid to phenolsulphonephthalein (sometimes more and sometimes less alkaline than $P_H - 7.2$) in spite of the fact that there was in all cases free and fairly rapid circulation visible through the capillaries. This indicates that the reaction of tissue cells may be on the acid side of neutral in spite of the fact that there is no actual asphyxia.

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A method for the volumetric study of the human hypophysis cerebri with illustrative results.

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The importance of the ductless glands in modern medicine has created a demand for more accurate data on these structures. Hammar of Upsala has particularly stressed this point. The need for quantitative facts is more urgent in the case of the hypophysis, or pituitary body, because this organ is composed of several parts which are more or less distinct structurally, functionally and embryologically; and because of the present tendency to classify pituitary disorders from the standpoint of hyper- or hyposecretion of each lobe, the time of onset with reference to

¹ Rowntree, L. G. and Geraghty, J. T., *J. Pharm. and Exper. Therap.*, 1910, i, 579.

² Rowntree, L. G. and Geraghty, J. T., *Arch. Int. Med.*, 1912, ix, 284.

adolescence, whether neoplastic or non-neoplastic, etc. The final justification for such a classification of the clinical pictures will depend, at least in a large measure, upon very careful post-mortem analysis of the hypophysis in large series of selected cases. But before pathological material can be properly evaluated it is necessary to establish something of a standard from normal cases, and to determine the range of variability of apparently healthy individuals.

Aside from the gross weight and the length of the three diameters, we have been unable to find on human material anything better than rough qualitative statements and these are greatly at variance with each other. This is particularly true of the relative number and arrangement of the three types of cells in pars anterior and in the amount of colloid. We know of no accurate volumetric data on the size of the various lobes in the hypophysis of man. We have, therefore, set out to determine the relative and absolute weight of the three principal parts of the main body of the gland and of the large colloid masses, together with the relative number and arrangement of the three types of cells in pars anterior of normal adult males between 20 and 60 years of age. For this material we are greatly indebted to the department of pathology, University of Minnesota.

Having made sufficient determinations to establish a comparatively simple routine method applicable to material obtained during regular autopsies, we are summarizing the method that others may profit by our efforts.

METHOD.

After trying various fixing fluids, we found that none were better than ordinary formalin, 15 to 30 per cent. in strength. If detailed work on the cytoplasmic granules is contemplated, the formalin may be neutralized with magnesium carbonate and the tissue chromated after the preliminary formalin fixation. For all general purposes, chromation is unnecessary. The advantage of formalin is that it not only admits of a sharp differential staining of the cells but produces practically no change in the weight of the hypophysis for several hours and but little modification even after several days. It is therefore possible to get the weight

of the fresh organ from formalin material, without the necessity of having delicate balances at the autopsy and without delaying fixation.

To avoid injury to the posterior lobe, it is safer to remove the entire sella turcica with the pituitary *in situ* by pinching off the clinoid processes and plunging the entire mass into formalin for later removal of the hypophysis. As soon as possible the excess dural sheath is removed and the infundibular stalk cut off close to the main body of the gland. The organ is again placed in formalin a few moments to moisten the outer surface which becomes somewhat dry as a result of the handling. After blotting off excess formalin the organ is weighed and then fixed three or four days longer in fresh formalin. Without washing in water, the tissue is dehydrated as usual, cleared in xylol and embedded in hard paraffin (60° C.), reducing the duration in the paraffin both to four or five hours by changing the paraffin at least three times.

The organ is cut horizontally, *i.e.*, through its greatest diameter, and not sagittally as in the prevailing method. If one is to depend upon a limited number of sections for an estimate of the condition of the gland, the view obtained in the horizontal plane is by far the best. Mid-sagittal sections are particularly atypical of pars anterior for this region is frequently very poor in eosinophiles. Sections are cut 10 μ till one fourth through the block when an even number (about 10) of 5 μ sections are cut for cytological work and differential cell counting. Cutting at 10 μ is resumed until the middle of the block is reached and again till three-fourths through when similarly a few 5- μ sections are obtained. These division points may be determined near enough by marking the end of the paraffin block after it is trimmed for sectioning.

The whole series is marked off into groups of twenty sections (two 5- μ sections counting as one) and from the middle of each group a 10- μ section is taken. These (usually 30 to 40 in number) can be mounted on three or four slides and stained with Mallory's connective tissue stain (acid fuchsin-aniline blue-orange G) as regularly done in staining connective tissue. Another similar set is taken from points midway between the other series and stained with hematoxylin and eosin in order to have two series

which together will constitute a more complete set consisting of every tenth section and also to have a better nuclear stain. Every section of one of these series only (as a rule) is projected at a magnification of twenty diameters upon "American Linen Record" paper (sheets 23×36 inches, 72 lbs. per ream) which runs very uniformly at .012 gram per sq. cm. Every sheet needs checking up, however. This is done by cutting out from each corner a square 5 cm. each way and weighing the four squares together. If a heavy sheet is balanced with a light sheet (several sheets being necessary for each determination) the weight per sq. cm. can be kept sufficiently constant. The capsule (including the connective tissue extending in between the lobes), pars anterior, connective tissue trabeculae and large colloid masses in pars anterior, parenchyma of pars intermedia, colloid in pars intermedia, and pars nervosa are outlined with a hard sharp pencil or with ink. Where the colloid and parenchyma of pars intermedia are very irregularly distributed, it is safer to use both series, thus reducing the error greatly.

These areas are cut out with scissors, using a fine manicuring scissors for cutting out the smaller areas and areas rounded by many sharp turns. The paper representing any particular part is weighed. The percentage that this weight is of the weight of the paper of all parts together constitutes the percentage of the whole organ represented by that part, if the shrinkage is the same for all parts in any particular case.

To determine the shrinkage of the two lobes, they were separated from each other in three cases and each lobe weighed before fixation. The members of each pair were kept together through the entire process of fixation, embedding, etc. From serial sections, as explained above, the final volume was obtained by dividing the total paper weight of a lobe by the weight of one sq. cm. of paper, then dividing the results by the actual magnification (magnification in diameter squared) and finally multiplying by 200μ ($20 \times 10 \mu$) reduced to cm. (.02 cm.). The original volume (before fixation) was determined by dividing the weight by the specific gravity.

The results are tabulated in Table I, from which it is seen that there is sufficiently close agreement between the shrinkage of the

two parts in each case that it may be considered the same despite the great difference in structure.

TABLE I.

SHRINKAGE PRODUCED BY THE TECHNIQUE WHEN THE POSTERIOR AND ANTERIOR LOBES WERE SEPARATED FROM EACH OTHER.

Autopsy Number.	20-446.		20-450.		22-140.	
Lobe.....	Anterior.	Posterior.	Anterior.	Posterior.	Anterior.	Posterior.
Original volume, c.c.....	.3632	.1415	.3113	.1057	.2547	.1113
Final volume, c.c..	.2430	.0950	.1928	.0666	.1540	.0691
Shrinkage, c.c.1202	.0465	.1185	.0391	.1007	.0422
Per cent. of shrinkage.....	33.1	32.9	38.1	37.0	39.5	38.0

This is a method which has been used extensively for similar purposes in this and other laboratories and especially by Godlewski, Hammar and Jackson, and the senior author has used it on the hypophysis of the woodchuck. Whether any time would be saved by measuring the areas with a planimeter has not yet been determined on this material. Dr. C. M. Jackson, of this laboratory, after comparing the two methods, favored the cutting-out process. The planimeter cannot increase the accuracy providing one has dexterity with the scissors.

For a differential count of the types of cells in pars anterior, a 5- μ section from each of the three planes mentioned is stained with Mallory's connective tissue stain, going rapidly through the alcohols following the stain. No better differential stain could be wished than this will give on material only a few hours post mortem. These three sections are systematically explored with an oil-immersion lens by means of a mechanical stage, and the cells of each type in each fifth field of each fifth row (as shown in Fig. 1) are counted and the percentage of the total calculated.

RESULTS.

In Table II are recorded some selected cases to illustrate what the method yields in four normal males with hypophyses covering the usual range in weight met with, one female with a rather large hypophysis and one case of non-neoplastic post-adolescent hypopituitarism of both lobes. The small normal male hypo-

physis (Nos. 21-66) is seen to have an especially small pars nervosa (posterior lobe). The great size of the female hypophysis is due to a large pars anterior. The hypopituitary case has a hypophysis very similar in volumetric relations to the female. The absence of data on the parenchyma of pars intermedia of the female hypophysis is due to the fact that the cells of this portion in this particular case were very few and the tissue not fresh enough when fixed to be well differentiated by the stain. This will have to be expected in a few cases since pars intermedia constitutes such a small irregular part of the entire gland—a fact of

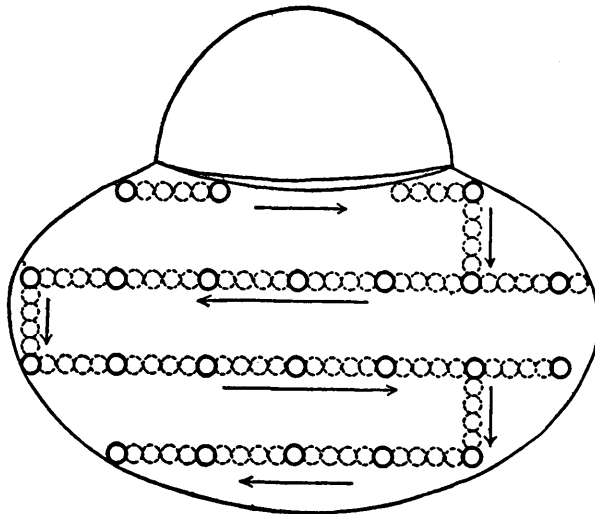


FIG. 1. Diagram showing how the field is explored in making the differential¹ count of cells in pars anterior. The solid circles represent the fields which are actually used.

some consequence in view of Bab's theory that the polyurea in hypopituitarism is due to hyposecretion of the pars intermedia and because in some of the lower forms this part of the hypophysis is distinctly connected with pigmentation. The greatest error will always be in connection with the juxtaneural portion; but the error can be decreased by placing this part of the gland under higher magnification if that should be deemed necessary.

In Table III is shown a typical cell count as obtained by the method here outlined.

TABLE II.
WEIGHT AND PERCENTAGE OF THE VARIOUS PARTS OF THE HUMAN HYPOPHYSIS.

Cases.	Autopsy Number..	20-323.		20-380.		21-68.		21-66.		21-14.		22-81.	
	Age.....	36 yr.	Male.	37 yr.	Male.	50 yr.	Male.	58 yr.	Male.	53 yr.	Female.	59 yr.	Male. ¹
Weight of Whole Gland (grams).....													
Pars Anterior (Distalis)	Sex.....	.530		.660		.585		.420		.830		.620	
	Trabeculae .	grams. .0043	% .84	grams. .0032	% .49	grams. .0099	% 1.70	grams. .0072	% 1.71	grams. .0159	% 1.92	grams. .0022	% .34
	Parenchyma	.3832	72.30	.4501	68.20	.3743	63.99	.2859	68.08	.6339	76.37	.4678	75.45
	Total.....	.3875	73.14	.4533	68.69	.3843	65.69	.2931	69.79	.6498	78.29	.4700	75.79
Pars Nervosa (Proc. Infund.) or Posterior.....													
Pars Intermedia (Juxtaneuralis)	Colloid.....	.0046	.86	.0022	.33	.0055	.94	.0036	.86	.0093	1.12	.0101	1.63
	Parenchyma	.0022	.42	.0110	1.67	.0104	1.77	.0040	.95	?	?	.0074	1.20
	Total.....	.0068	1.28	.0132	2.00	.0159	2.71	.0076	1.81	?	?	.0175	2.83
	Capsule.....	.0234	4.42	.0389	5.90	.0259	9.05	.0471	11.21	.0460	5.54	.0386	6.22

¹ A typical specimen of so-called non-neoplastic post-adolescent hypopituitarism of both lobes (diagnosis by Dr. Wm. O'Brien). 189 cm. long. Weight 330 lbs. 3 hrs. post mortem.

TABLE III.

DIFFERENTIAL COUNT OF THE CELLS IN PARS ANTERIOR OF HYPOPHYSIS FROM
A 63-YR.-OLD WOMAN (AUTOPSY NUMBER 21-190—3 HR. POST MORTEM).

Total fields from three different levels 150. Sections 5μ thick. Formalin fixation. Mallory's C.T. Stain.

Cell Types.	All Types Together.	Chromophobes.	Acidophiles.	Basophiles.
Number of cells counted . .	25,658	14,776	8,510	2,372
Percentage	100	57.6	33.2	9.2

ABSTRACTS OF THE COMMUNICATIONS.

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The effect of thyroidectomy on the intelligence of sheep.

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The procedure followed in attempting to determine the effect of thyroidectomy on the intelligence of sheep has already been described.¹ Four pairs of twin female lambs from three to four weeks old were caused to learn a labyrinth with a single cul de sac and the twin of each pair making the better record was thyroidectomized by Dr. Simpson. Two months later the operated lambs had become typical cretins.

One hundred two days following thyroidectomy one of the cretins, already definitely lethargic, and her normal twin began relearning the labyrinth. The position of the cul de sac was then reversed and the labyrinth was again learned. Learning was taken to be complete at the end of three trials without error; an error being counted whenever the animal entered the cul de sac or turned back along the true path. The number of steps taken

¹ Liddell H. S., PROC. SOC. EXPER. BIOL. AND MED., 1922, xix, 343.