EFFECTS OF INANITION AND REFEEDING UPON THE GROWTH AND STRUCTURE OF THE HYPOPHYSIS IN THE ALBINO RAT

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FIVE FIGURES

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On account of the intimate relations usually assumed to exist between the hypophysis and the general growth process of the body, the effects of inanition and refeeding upon this organ have unusual interest and importance. Few observations upon these effects are recorded in the literature. In previous papers (Jack-

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son '15 a; '15 b) it was shown that, in young rats held at maintenance (constant body weight) by underfeeding, the hypophysis usually increases slightly in gross weight; while in adult rats with acute or chronic inanition the weight of the hypophysis decreases nearly in proportion to that of the whole body. The purpose of the present paper is to present the results of an extension of the investigation, including a volumetric and histological study of the various parts of the hypophysis.

This paper forms the fourth of a series of studies upon the effects of inanition on the albino rat, the investigation being supported by a special grant from the research funds of the Graduate School of the University of Minnesota.

MATERIAL AND METHODS

The material used included the hypophyses from 91 rats, obtained partly in connection with my previous studies (Jackson '15 a and '15 b) and partly from material collected by Hoskins ('16) and Stewart ('16). The 91 rats included 44 normal (control) rats of both sexes, varying from newborn to about one year of age; 15 rats held at maintenance (constant body weight) by underfeeding beginning at the age of three weeks; 6 older rats subjected to acute inanition and 5 to chronic inanition; and 21 young rats refed for various periods after being held at maintenance from the age of three weeks to ten weeks or more.

The diet in all cases was whole wheat (graham) bread soaked in whole milk, the amount being reduced for maintenance and chronic inanition, and cut off entirely in acute inanition. Water was supplied *ad libitum* in all cases. The loss in body weight during acute and chronic inanition (adults) was about one-third.

The general data for the rats used are given in table 1. In the first column, the letter indicates the series ('H' = Hoskins; 'St.' = Stewart, etc.). The number preceding the decimal point is the litter number; the number following designates the individual rat. (This does not apply to most of the rats in subdivisions 'C' and 'D' of table 1, however, where the litters were not recorded.)

TABLE 1

General data for albino rats used

RAT NO.	SEX	AGE	NOSE-ANUS LENGTH	BODY WEIGHT GROSS (AND NET)	HYPOPHYSIS WEIGHT (FRESH)
A. Normal rats					
		days	*mm.*	*grams*	*gram*
J 1.7a	m	Newborn		4.9	
J 1.7b	m	Newborn		4.9	
St 32.1	f	½	46	5.0	
St 72.2	m	7	66	10.8(10.0)	0.0013
St 72.5	f	7	66	10.8(10.1)	0.0012
St 80.5	f	12	75	15.5	0.0020
St 80.7	m	12	75	15.0	0.0018
J 1.2	f	21	100	28.2	0.0018
J 1.1	m	21	100	29.0	0.0020
S 7.29	f	21	107	31.5(30.4)	0.0022
S 47.1	f	21	101	33.4	0.0018
St 5.1	f	56		63.0	0.0040
St 47.6	f	67	169	123.5	0.0069
St 47.5	m	67	191	196.0	0.0065
H 70.3	m	70	194	208.1(198.3)	0.0078
H 70.7	f	71	184	162.7(155.0)	0.0099
S 11.62	f	72	170	119.3(115.2)	0.0046
H 68.11	m	72	192	170.8(163.8)	0.0068
H 68.8	f	74	184	141.6(137.0)	0.0088
H 68.3	f	74	185	158.5(151.3)	0.0084
M 1.2	m	74	190	181.1(172.6)	0.0063
S 5.4	f	74	168	125.9(117.6)	0.0050
S 5.3	m	74	180	172.0(166.5)	0.0067
H 60.3	f	78	182	138.2	0.0077
J 1.3	m	94	183	177.0	0.0063
H 65.7	f	98	179	144.1(138.9)	0.0084
H 64.3	m	101	191	184.7(179.2)	0.0071
H 59.3	f	103	196	(165.6)	0.0097
H 63.2	m	103	195	173.7(166.1)	0.0079
H 58.3	m	106	225	(258.1)	0.0089
St 12.54	f	111	166	121.0(115.9)	0.0058
St 10.24	f	112	169	134.5(129.3)	0.0099
J 1.7[1]	f	112	185	161.0	0.0092
H 50.5	f	132	196	(173.3)	0.0141
H 36.3	m	138	202	(201.6)	0.0089
H 50.3	m	141	211	(221.5)	0.0088
H 47.3	m	145	201	(167.6)	0.0060
H 34.3	f	224	194	(173.0)	0.0123
H 34.6	f	225	205	(187.5)	0.0108

[1] At end of pregnancy.

TABLE 1—Continued

RAT NO.	SEX	AGE	NOSE-ANUS LENGTH	BODY WEIGHT GROSS (AND NET)	HYPOPHYSIS WEIGHT (FRESH)
		days	*mm.*	*grams*	*grams*
H 24.3	f	232	188	(138.4)	0.0077
H 27.3	f	253	195	(166.4)	0.0121
St 9.48	f	338	195	205.0(197.8)	0.0196
St 33.116	f	340	195	195.5(188.3)	0.0108
St 8.36	m	351	228	381.0(369.3)	0.0088
B. Rats held at maintenance from age of three weeks					
St 47.3	m	66	120	34.0	0.0022
St 47.4	m	66	113	32.3	0.0020
S 12.69	f	66	100	24.5(22.7)	0.0020
S 12.71	m	67	95	23.3(21.2)	0.0020
S 7.31	m	70	120	34.8(31.4)	0.0018
S 9.36	m	72	113	30.5	0.0018
S 6.23	f	72	102	26.1(24.5)	0.0033
S 11.63	f	72	95	23.8(22.6)	0.0022
S 5.8	f	73	105	27.6(24.6)	0.0011(?)
S 5.11	f	73	105	25.3(23.3)	0.0018
S 11.65	m	73	100	23.8(22.5)	0.0022
S 5.12	f	74	103	26.1(25.5)	0.0017
St 12.50	m	82	123	45.0(41.2)	0.0023
St 33.1	f	104	89	19.1(18.2)	0.0019
St 38.8	m	139	118	30.0	0.0020
C. Rats subjected to acute inanition					
J 1.4	m	94	180	107.0	0.0056
M 1	m	?	205	168.0(165.2)	0.0070
M 2	m	?	?	170.0(167.2)	0.0066
S 26	m	?	205	174.0(171.5)	0.0076
S 16	f	?	195	190.0(186.0)	0.0146
S 27	m	?	215	223.0(219.0)	0.0096
D. Rats subjected to chronic inanition					
J 1.5	m	117	175	97.0	0.0042
M 12	m	?	173	128.0(124.6)	0.0052
M 5	m	?	190	129.0(126.3)	0.0058
M 6	m	?	175	138.0(134.1)	0.0060
M 11	m	?	190	163.0(158.5)	0.0064
E 1. Rats refed one-half week after maintenance (three to twelve weeks)					
St 12.53	m	85	123	48.5(44.9)	0.0028
St 10.25	f	87	113	40.5(36.5)	0.0022

TABLE 1—Concluded

RAT NO.	SEX	AGE	NOSE-ANUS LENGTH	BODY WEIGHT GROSS (AND NET)	HYPOPHYSIS WEIGHT (FRESH)
E 2. Rats refed one week after maintenance (three to twelve weeks)					
		days	*mm.*	*grams*	*grams*
St 11.41	m	88	132	62.2(58.2)	0.0031
St 11.42	f	88	125	55.0(50.3)	0.0031
St 11.45	f	88	130	67.6(57.7)	0.0034
St 12.48	f	88	125	50.2(46.5)	0.0028
St 10.27	f	89	127	55.0(51.4)	0.0028
E 3. Rats refed two weeks after maintenance (three to twelve weeks)					
St 11.40	f	95	142	84.5(76.5)	0.0030
St 11.43	f	95	143	79.0(74.7)	0.0034
St 12.49	f	95	129	60.5(55.5)	0.0027
St 12.51	f	95	137	77.2(70.7)	0.0034
St 10.26	m	96	150	91.0(86.3)	0.0033
E 4. Rats refed four weeks after maintenance (three to twelve weeks)					
St 12.52	m	109	171	125.2(117.4)	0.0056
St 12.47	f	109	161	106.5(98.0)	0.0082
St 11.44	f	109	151	94.2(88.4)	0.0058
St 11.46	f	109	163	118.0(110.1)	0.0088
St 10.28	f	110	167	118.0(109.5)	0.0068
St 10.23	f	111	168	129.5(119.1)	0.0094
E 5. Rat refed one year after maintenance (three to ten weeks)					
S 14.3	m	444	236	332.0(318.9)	0.0097
E 6. Rats refed after maintenance from age of three to twenty weeks					
S 33.120	f	339	181	162.0(155.9)	0.0096
S 33.118	m	346	204	229.0(218.5)	0.0094

The material was obtained immediately after the animals were killed (by chloroform). Especial care must be taken to avoid injury in the removal of delicate organs like the hypophysis. The glands were in nearly all cases fixed in Zenker's fluid and stained with haematoxylin-eosin. Formalin and Zenker-formol were tried as fixatives, and iron-haematoxylin as a stain, with less satisfactory results. The glands were embedded in paraffin, and cut in sections (usually serial) 3 μ to 5 μ in thickness. The sections were cut in the frontal (coronal) plane.

The volumes of the various parts (lobes) of the hypophysis were determined by a method similar to that used by Hammar ('14) for the thymus. The outlines of the sections, magnified 75 diameters, were projected upon "American Linen Record" paper (sheets 18 x 23 inches, 36 lbs. per ream) by means of an Edinger projection apparatus. Four samples, each 5 cm. square, were weighed from each sheet, and the area corresponding to each gram of paper determined. The various lobes as outlined were then cut out and weighed, and the corresponding areas calculated. This magnified area was then reduced to actual area and multiplied by the thickness of the sections, giving the actual volume for each lobe. By trial it was found to be unnecessary to draw every section, about 50 sections taken at equal intervals (every fifth to tenth section) being found to give nearly identical results.

On comparing the original weight of the hypophysis with the volume obtained by the preceding method, there is apparently a marked discrepancy, the volume (in cubic centimeters) being less than half the weight (in grams) (table 2). The difference is due: (1) to the density of the gland; (2) to the capsule and extracapsular structures attached, which were weighed but not included in the volume measured; (3) to the great shrinkage resulting from the process of fixation, dehydration and embedding in paraffin. This process alone would probably account for a shrinkage of nearly one-third in volume.

For the pars anterior (distalis), a plan similar to that above given for the lobes was followed to determine the relative volumes of the vascular stroma and the parenchyma; and of the nuclei and cytoplasm in the parenchyma. For this purpose, a higher magnification (Zeiss 2 mm. apochromat with compensating ocular 6, giving magnification of 1420 diameters at table level) was used. A typical field was chosen, and as large an area as possible drawn, with the aid of a Spencer camera lucida. It is important that the section drawn represent as nearly as possible a true optical plane, and therefore no change of focus during the drawing is permissible. If this is not carefully done, there will be a tendency to draw the nuclei too large, since their maximum

	NOSE-ANUS LENGTH	BODY WEIGHT	HYPOPHYSIS WEIGHT	PARS ANT.	PARS INT.	PARS NERV.
	mm.	*grams*	*grams*	*per cent*	*per cent*	*per cent*
Males	190	188	0.00685	82.0	9.7	8.3
Females	179	148	0.00808	86.4	6.7	7.0

Thus in the females the pars anterior appears to have gained, while the pars intermedia and, to a lesser extent, the pars nervosa, have decreased in relative (percentage) weight.

The question naturally arises as to whether the increased relative size of the anterior lobe in the female is sufficient alone to account for the known sexual difference in the weight of the gland. In the averages above shown, the higher percentage weight in the hypophysis of the female is more than sufficient to account for the sexual difference in the gross weight of the gland upon this basis. But this disregards the fact that in these cases the body weight is higher in the males, which might alter the relations. If, however, we compare female H 70.7 and male H 68.11, whose ages and body weights are not very different (see table 1), we still find that the larger hypophysis of the female (0.0099 gram) as compared with that of the male (0.0068 gram) may be accounted for as due chiefly to the heavier anterior lobe in the former (86.2 per cent by volume, as against 80.7 per cent in the male), as shown in table 2. Or, comparing the absolute volumes (not given in the table), in the hypophysis of the male H 68.11 the volumes of the partes anterior, intermedia and nervosa were 0.00241 cc., 0.00033 cc. and 0.00025 cc., respectively; while in female H 70.7 the corresponding volumes were 0.00386 cc., 0.00034 cc. and 0.00029 cc. Thus the larger hypophysis of the female showed but slight increase in the partes intermedia and nervosa, but a very large increase in the pars anterior.

It should further be noted, however, that in the rats used the sexual difference in the weight of the hypophysis is not so great as that shown in Donaldson's ('15) tables for rats of corresponding weight or body length. To produce this difference would require a greater preponderance of relative size for the anterior lobe of the female than is found in my measurements. It is

TABLE 2

Volumetric data on the parts (Lobes) of the hypophysis of the albino rat under various conditions

RAT NO.	SEX	AGE	GROSS BODY WEIGHT	HYPOPHYSIS WEIGHT	VOLUME OF GLAND	VOLUME OF LOBES	PERCENTAGE FORMED BY: Pars anterior	Pars intermedia	Pars nervosa
A. Normal rats (controls)									
		days	*grams*	*grams*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*
J 1.7a	m	Nb	4.9		0.0001745	0.0001599	77.7	10.5	11.5
J 1.1	m	21	29.0	0.0020	0.0006494	0.0006480	76.9	8.0	15.0
St 47.5	m	67	196.0	0.0065	0.003172	0.003085	85.3	6.0	8.7
H 70.3	m	70	208.1	0.0078	0.003662	0.003662	79.8	11.9	8.3
H 68.11	m	72	170.8	0.0068	0.003068	0.002993	80.7	11.0	8.3
J 1.3	m	94	177.0	0.0063	0.002844	0.002818	82.3	9.7	8.0
J 1.2	f	21	28.2	0.0018	0.0007877	0.0007597	79.2	8.0	12.0
S 47.1	f	21	33.4	0.0018	0.0008064	0.0007913	81.0	6.0	13.0
St 47.6	f	67	123.5	0.0055	0.002946	0.002868	86.9	6.0	7.4
H 70.7	f	71	162.7	0.0099	0.004598	0.004485	86.2	7.5	6.5
J 1.7*	f	112	161.0	0.0092	0.004346	0.004300	87.0	5.7	7.3
H 24.3	f	232	145.0	0.0077	0.003685	0.003568	85.4	7.7	6.9
B. Rats held at maintenance from age of three weeks									
St 47.4	m	66	32.3	0.0020	0.0008340	0.0008199	76.1	7.9	15.9
St 47.3	m	66	34.0	0.0022	0.0008609	0.0008417	75.2	9.0	16.0
St 12.50	m	82	45.0	0.0023	0.001053	0.001053	71.0	10.5	18.5
C. Adult rats with acute inanition									
S 27	m		223.0	0.0096	0.003957	0.003900	84.4	6.5	9.0
S 16	f		190.0	0.0078	0.005723	0.005665	89.6	3.5	6.9
D. Adult rats with chronic inanition									
M 12	m		128.0	0.0052	0.002491	0.002467	79.0	7.0	14.0
E 1. Rats refed one-half week after maintenance from three to twelve weeks of age									
St 10.25	f	87	40.5	0.0022	0.001080	0.001060	75.6	7.0	17.5
E 2. Rats refed one week after maintenance from three to twelve weeks of age									
St 11.41	m	87	62.2	0.0031	0.001558	0.001462	78.3	8.0	13.7
St 10.27	f	89	55.0	0.0028	0.001534	0.001494	81.0	7.0	12.0
St 11.45	f	88	67.6	0.0034	0.001431	0.001405	82.0	7.0	11.0

* Rat No. J 1.7 had just given birth to a (first) litter.

TABLE 2—Concluded

RAT NO.	SEX	AGE	GROSS BODY WEIGHT	HYPOPHYSIS WEIGHT	VOLUME OF GLAND	VOLUME OF LOBES	PERCENTAGE FORMED BY Pars anterior	Pars intermedia	Pars nervosa
E 3. Rats refed two weeks after maintenance from three to twelve weeks of age									
		days	*grams*	*gram*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*
St 10.26	m	96	91.0	0.0033	0.001843	0.001803	78.6	7.0	14.2
St 11.40	f	95	84.5	0.0030	0.001496	0.001443	81.6	6.0	12.4
St 11.43	f	95	79.0	0.0034	0.001680	0.001643	79.0	7.0	14.0
St 12.51	f	95	77.2	0.0034	0.001362	0.001347	79.2	6.8	14.0
E 4. Rats refed four weeks after maintenance from three to twelve weeks of age									
St 12.47	f	109	106.5	0.0082	0.002608	0.002608	86.7	4.5	8.8
E 5. Rats refed six or seven months after maintenance to age of about five months									
S 33.118	m	346	229.0	0.0094	0.004155	0.004086	78.0	6.7	15.2
S 33.120	f	339	162.0	0.0096	0.003981	0.003927	78.1	7.0	14.0

therefore possible that further data would modify my provisional conclusion that the larger gland in the female is due to a larger anterior lobe.

The pars intermedia averages somewhat higher in the males than might be expected according to the theory above mentioned (that the sexual difference in hypophysis weight is due to difference in the anterior lobe alone), but this is probably due to the unusual individual variations, which table 2 shows to occur in the pars intermedia.

It may be noted that in the one rat (J 1.7) killed at the end of pregnancy, the expected hypertrophy of the gland apparently failed to occur, and no marked change is evident in the relative size of the three parts (lobes). The other females used were all virgins.

b. Volumes of the lobes in young rats held at maintenance

As shown in table 2 B, in the 3 young rats held at maintenance (nearly constant body weight) from three to ten or twelve weeks, the pars anterior has been reduced in relative volume, the partes intermedia and nervosa becoming correspondingly larger. This change is especially well marked in the rat held at maintenance up to twelve weeks of age.

c. Volumes of the lobes in adult rats subjected to acute and chronic inanition

The 2 rats, S 27 and S 16, had been subjected to acute inanition (water but no food given) for about ten days, with resultant loss of about 30 per cent in body weight. The final body weights are given in table 1. The pars anterior of the hypophysis in these rats appears relatively somewhat larger than normal, the pars intermedia somewhat smaller, the pars nervosa not much changed in relative size (table 2 C).

In the rat (M 12) subjected to chronic inanition (progressive underfeeding for thirty-six days, with loss of 36 per cent in body weight), the partes anterior and intermedia are smaller and the pars nervosa larger than normal in relative size (table 2 D). Thus the relative changes in the size of the lobes during chronic inanition of the adult appear in general to resemble more nearly the changes in the young during maintenance, which are somewhat different from those during acute inanition. The general resistance of the pars nervosa during inanition recalls the similar behavior of the closely related brain.

d. Volumes of the lobes in rats refed after maintenance

As seen in table 2 (E 1, E 2, E 3 and E 4), in the young rats refed one-half week, one week, two weeks and four weeks, there is a considerable individual variation in the relative size of the parts (lobes) of the hypophysis. In general, however, there appears a gradual return to the normal proportions, the pars anterior increasing and the partes intermedia and nervosa decreasing in relative (percentage) volume. The exceptionally small pars intermedia in the rat refed 4 weeks is probably an individual variation.

Two rats (table 2 E 5) were refed six or seven months after a long period of maintenance, from the age of three weeks to five months. In these cases, although the body weights and hypophysis weights are nearly normal, the lobes are abnormal in their relative size. In each the pars anterior is relatively small, and the pars nervosa large. This resembles the change found after maintenance in young rats and chronic inanition in older rats. It is therefore probably a persistent effect of the inanition during the prolonged period of maintenance.

VOLUMETRIC ANALYSIS OF TISSUES AND CELLS IN PARS ANTERIOR

As shown in table 3, data were obtained in eight cases by the method previously described. A larger number of observations

TABLE 3

Volumetric data on the component parts (tissues and cells) in the pars anterior (distalis) of the hypophysis of the albino rat under various conditions.

RAT NO.	SEX	AGE AND CONDITION	VESSELS AND STROMA	PAREN-CHYMA	PERCENTAGE OF PARENCHYMA FORMED BY		PARENCHYMA CELLS AVERAGE DIAMETER (CALCULATED)	
					Nuclei	Cyto-plasm	Nuclei μ	Cell μ
			per cent	per cent	per cent	per cent		
J 1.7a	m	Normal newborn	6.7	93.3	34.1	65.9	5.9	10.1
St 47.1	f	Normal 21 days	9.6	90.4	24.1	75.9	5.8	11.9
St 47.5	m	Normal 67 days	10.6	89.4	19.7	80.3	6.0	13.6
S 5.12	f	Maintained 3–10 weeks	13.4	86.6	25.9	74.1	5.0	9.7
S 6.23	f	Maintained 3–10 weeks	8.8	91.2	23.4	76.6	4.9	10.2
St 33.1	f	Maintained 3–15 weeks	13.4	86.6	28.4	71.6	5.3	10.0
S 27	m	Adult acute inanition	16.7	83.3	25.7	74.3	5.5	10.8
M 12	m	Adult chronic inanition	17.5	82.5	23.1	76.9	5.3	11.0

would doubtless reveal much individual variation, so that no great emphasis can be laid upon the exact figures in the data obtained. These quantitative data confirm the general impressions noted in the much larger number of cases studied, however, so that the general conclusions may be regarded as fairly certain.

a. Relative volumes of stroma and parenchyma (table 3)

In the pars anterior of the normal newborn rat, the vessels and associated stroma form 6.7 per cent of the total volume, increasing to 9.6 per cent at three weeks and to 10.6 per cent at ten weeks (sixty-seven days). At this time the gland appears to have reached its maximum normal vascularity; though no actual measurements were made upon later stages, and there is naturally some individual variation.

In young animals held at maintenance, there is usually a striking increase in the vascularity, the sections showing a marked hyperemic appearance. This is confirmed by the measurements on two of the three cases in table 3, both showing an increase of the vascular stroma to 13.4 per cent. The third case represents an unusual condition in which, on the other hand, there appears to be a slight decrease to 8.8 per cent in the volume of the vessels and associated stroma.

In adult rats subjected to acute or chronic inanition the hyperemia is usually even more conspicuous than in the younger animals. This is confirmed by the measurements, showing for the vascular stroma 16.7 per cent by volume for the case of acute inanition and 17.5 per cent for the chronic inanition. These may be considered as typical, though here also individual variations occur.

In general, therefore, it appears that during inanition in the pars anterior the parenchyma becomes reduced in relative volume, with corresponding increase in the vascular stroma. This increase is due chiefly to a distention of the blood-vessels, giving the sections a markedly hyperemic appearance. In a few cases, especially in the young held at maintenance, there is also some increase in the intercellular substance.

b. Relative volumes of the nuclei and cytoplasm of the parenchyma (table 3)

In the parenchyma of the pars anterior, the nuclei form 34.1 per cent of the total cell volume in the newborn, decreasing to 24.1 per cent at three weeks and to 19.7 per cent at ten weeks (sixty-seven days). The cytoplasm, of course, undergoes a corresponding increase in relative volume (figs. 2 and 4). The relations found in the specimen at ten weeks (St 47.5) appear to be typical for adults, although no actual measurements were made at later stages. Some allowance must also be made for variations, both in different individuals and in different parts of the gland in the same individual.

In 2 of the 3 young rats held at maintenance, in which the volumetric data were obtained, there appears to be a relative increase in the nuclear volume to 25.9 per cent and 28.4 per cent respectively. This involves a corresponding loss in relative volume for the cytoplasm, which is doubtless typical (fig. 3). Individual variations occur, however, as in the third case (S 6.23, which also had a small stroma volume), in which the nuclei formed 23.4 per cent by volume, or slightly less than the normal at three weeks.

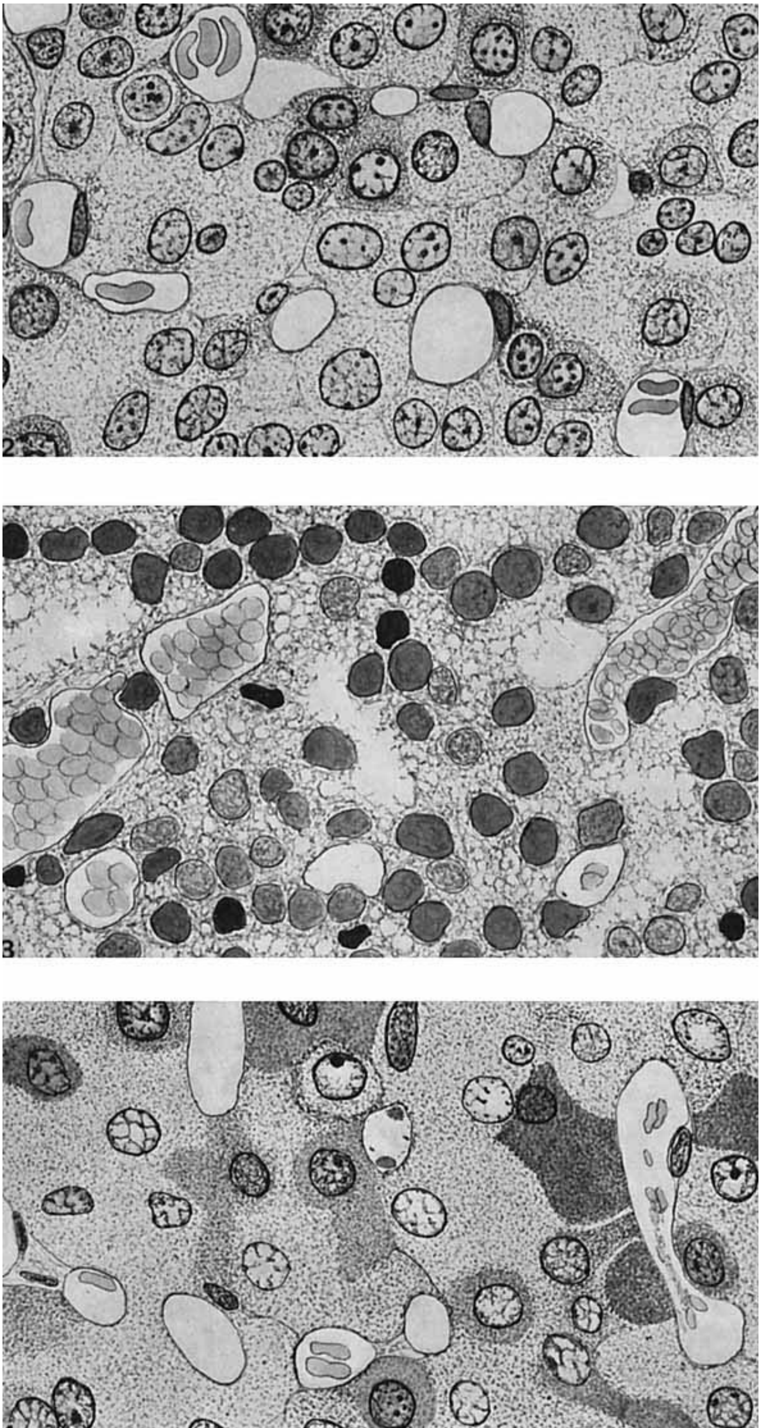
In the two older rats subjected to chronic and acute inanition the relative increase in nuclear volume (with corresponding cytoplasm decrease) appears even more strongly marked, reaching 23.1 and 25.7 per cent respectively, in comparison with 19.7 per cent for the normal. The crowding of the nuclei with reduction of cytoplasm is evident in figure 5. Allowance must of course be made for variations, but the general tendency is unmistakable.

c. Estimated size of cells and nuclei

The average diameters of the cells and nuclei of the parenchyma in the pars anterior, calculated as explained under "Material and Methods," are given in the last two columns of table 3. The average cell diameters by this method appear to increase from 10.1 μ in the newborn to 11.9 μ at three weeks and 13.6 μ at ten weeks (sixty-seven days). This appears to represent the average permanent adult size, although individual variations occur.

In the young rats held at maintenance, the average cell diameters in the three cases measured by this method range from 9.7 μ 10.2 μ, or considerably less than the normal at three weeks (11.9 μ). In the adults subjected to acute and chronic inanition, the cell diameters are 10.8 μ and 11.0 μ, likewise a marked reduction in comparison with the normal (13.6 μ).

The corresponding average nuclear diameters, estimated by this method, show very little change with age in the normal rats,


2
3
4

being 5.9 μ in the newborn, 5.8 μ at three weeks and 6.0 μ at ten weeks (sixty-seven days). In the three young rats held at maintenance, the nuclear diameter has diminished, varying from 4.9 μ to 5.3 μ. In the 2 older starved rats the corresponding diameters are 5.3–5.5 μ.

DIRECT MEASUREMENT OF NUCLEAR DIAMETERS

Owing to the imperfections of the volumetric method previously considered, and to the small number of cases to which it was applied, the results should be considered as only approximate and not final. So far as the nuclear diameters are concerned, however, the results have been controlled by a totally different method, that of direct measurement of the nuclear diameters with a filar micrometer eyepiece. Where the nuclear diameters are unequal (slightly ellipsoidal forms are frequent), the average of the longer and shorter diameters was taken. Nuclei apparently cut near the edge, so as to leave the maximum extent outside the section, were avoided so far as possible. The average (and range) for 100 nuclei in each case is shown in table 4.

The results of these direct nuclear measurements are more uniform, and in closer agreement with those of the volumetric method, than might be expected. This will appear upon a com-

Fig. 2 A small portion of the pars anterior of the hypophysis in a normal rat at three weeks (J 1.2). Most of the cells are of the faintly basophilic type. Some eosinophilic cells are indicated by darker staining (two near the upper margin of the figure). A few chromophobic cells are shown, a group of four near the right margin. Zenker fixation; haematoxylin stain. Drawn with the aid of a camera lucida. × 950.

Fig. 3 A small portion of the pars anterior of the hypophysis in a young rat (S 5.12) held at maintenance from age of three to ten weeks. The inanition effect is very marked, with hyperemia and atrophy of the parenchyma. Cytoplasm diminished in amount, sparsely granular and filled with coarse vacuoles which in places coalesce to form irregular spaces. Nuclei hyperchromatic, in various stages of pycnosis. Zenker fixation; haematoxylin-eosin stain. Drawn with the aid of a camera lucida. × 950.

Fig. 4 A small portion of the pars anterior of the hypophysis in a normal rat aged ten weeks (St 47.5). This represents the typical adult structure. Most of the cells are of the weakly basophilic type. Several eosinophiles are indicated, the more strongly acidophilic having a darker color. Zenker fixation; haematoxylin-eosin stain. Drawn with the aid of a camera lucida. × 950.

parison of tables 3 and 4. The direct nuclear measurements were made in a larger number of cases (12) and therefore give a somewhat better idea of the amount of individual variation.

The direct measurements of nuclei, as shown in table 4, were made for the pars intermedia as well as the pars anterior. Considering the latter first, measurements were in most cases made separately for the eosinophile and non-eosinophile (including

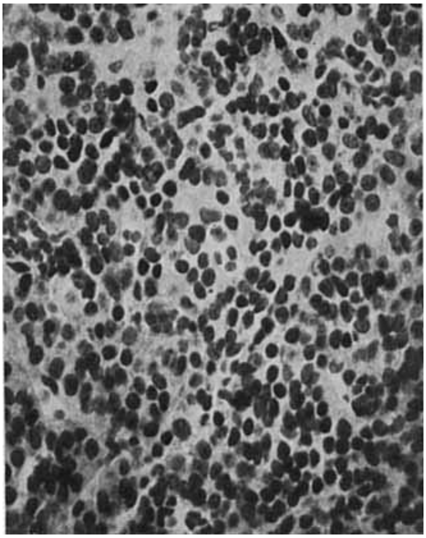


Fig. 5 From a photograph showing a small part of the anterior lobe of the hypophysis in an adult rat (M 12) subjected to chronic inanition. Marked effects of inanition: hyperemia; atrophy and loss of specific staining reactions in cytoplasm; hyperchromatism and pycnosis of nuclei. Zenker fixation; haematoxylin-eosin stain. × 440.

basophile and chromophobe) cells. In the earlier normal stages there does not appear to be any constant difference in nuclear size between the two classes of cells. But later, and especially in the rats subjected to inanition, the average is less for the nuclei of eosinophiles. This is probably because the eosinophiles appear more prone to karyopycnosis.

The nuclei of the pars intermedia cells do not differ much in size from those of the pars anterior, especially of the non-eosino-

TABLE 4

Average diameter of nuclei of parenchyma cells in the hypophysis of the albino rat under various conditions. Direct measurements by means of the filar micrometer. Average (and range) of 100 nuclei in each case

RAT NO.	SEX	AGE AND CONDITION	PARS ANTERIOR (DISTALIS)		PARS INTERMEDIA
			Non-eosinophiles	Eosinophiles	
			μ	μ	μ
J 1.7a	m	Normal newborn	[1]5.8(3.4–7.3)		5.8(3.4–7.5)
J 1.1	m	Normal 21 days	5.7(4.2–6.9)	5.8(4.5–6.8)	5.7(5.0–6.8)
St 47.1	f	Normal 21 days	5.6(4.0–7.0)	5.7(4.5–6.6)	5.4(4.0–7.0)
St 47.5	m	Normal 67 days	5.7(4.5–6.6)	5.7(4.8–6.3)	5.8(5.1–7.1)
St 47.6	f	Normal 67 days.	6.0(4.0–7.5)	5.7(3.8–6.7)	5.8(4.8–7.1)
H 34.6	f	Normal 225 days	5.9(4.7–6.7)	5.4(4.3–6.3)	6.1(5.0–7.2)
S 6.23	f	Maintained 3–10 weeks	5.6(4.2–6.9)	5.2(3.5–6.1)	5.4(4.4–7.2)
S 5.12	f	Maintained 3–10 weeks	[1]5.4(4.1–6.5)		5.4(4.4–6.2)
St 33.1	f	Maintained 3–15 weeks	[1]5.3(3.7–6.9)		5.8(4.5–6.6)
S 27	m	Adult acute inanition	5.3(4.0–6.4)	5.1(3.6–6.4)	5.5(4.5–6.6)
M 1	m	Adult acute inanition	5.2(3.5–6.2)	5.3(3.5–6.4)	5.7(4.3–6.9)
M 12	m	Adult chronic inanition	5.2(3.7–6.5)	4.7(3.1–5.7)	5.3(3.3–6.1)

[1] Including some eosinophiles.

philes. During inanition they show in most cases less shrinkage than do the pars anterior nuclei (especially of eosinophiles), though here as elsewhere much individual variation is found.

OCCURRENCE OF MITOSIS IN THE PARTS (LOBES)

In order to determine the rate of mitosis in the hypophysis of the rat under various conditions, the number of mitoses was counted in 40 cases, as shown in table 5. In each case, one or more (up to 5) entire coronal (frontal) sections, chosen to show maximum areas (especially of partes intermedia and anterior) were systematically examined by means of a mechanical stage, and the total number of mitoses counted for each part (lobe). As might be expected, much individual variation is found, both in different individuals and in different sections of the same gland. The general trend, however, is clear and is well shown in the condensed table of averages (table 6). Amitosis was not observed in any case.

TABLE 5

Mitosis in the hypophysis of the albino rat under various conditions

RAT NO.	SEX	AGE	GROSS BODY WEIGHT	HYPOPHYSIS WEIGHT	NUMBER OF MITOSES COUNTED IN ENTIRE CORONAL SECTIONS OF THE HYPOPHYSIS IN THE		
					Pars anterior	Pars intermedia	Pars nervosa
A. Normal rats							
		days	*grams*	*grams*			
J 1.7a	m	nb.	4.9		46; 68; 67; 66	8; 8; 13; 5	14; 4; 5; 6
St 72.5	f	7	10.8	0.0012	16; 15; 18; 23; 20	0; 0; 1; 1; 2	1; 1; 0; 0; 0
St 80.5	f	12	15.5	0.0020	16	0	0
J 1.1	m	21	29.0	0.0020	5; 7; 7; 11; 7	2; 3; 1; 2; 3	0; 0; 0; 0; 0
S 7.29	f	21	31.5	0.0022	7; 10; 8; 9; 5	1; 1; 1; 2; 2	0; 0; 0; 0; 0
S 47.1	f	21	33.4	0.0018	5; 6; 6; 6; 6	1; 0; 1; 0; 2	0; 0; 0; 0; 0
St 47.6	f	67	123.5	0.0055	2; 4; 5; 7; 2	0; 0; 0; 0; 0	0; 0; 0; 0; 0
St 47.5	m	67	196.0	0.0065	0; 0; 0	0; 0; 0	0; 0; 0
H 68.11	m	72	170.8	0.0068	2	0	0
S 5.3	m	74	172.0	0.0067	3; 2; 1; 1	0; 1; 0; 0	0; 0; 0; 0
J 1.3	m	94	177.0	0.0063	10; 12; 8	1; 1; 2	0; 0; 0
H 63.2	m	103	173.7	0.0079	0; 2	0; 1	0; 0
St 12.54	f	111	121.0	0.0058	0; 2	0; 0	0; 0
H 34.6	f	225	195.0	0.0108	0; 0; 1	0; 0; 0	0; 0; 0
B. Rats held at maintenance from age of three weeks							
St 47.3	m	66	34.0	0.0022	0; 0; 0; 1 ; 0;	0; 0; 0; 0; 0	0; 0; 0; 0; 0
St 47.4	m	66	32.3	0.0020	0; 0; 0; 1 ; 0	0; 0; 0; 0; 0	0; 0; 0; 0; 0
S 6.23	f	72	26.1	0.0033	0	0	0
S 5.11	f	73	25.3	0.0018	0	0	0
S 11.65	m	73	23.8	0.0022	0	0	0
S 5.12	f	74	26.1	0.0017	0	0	0
St 33.1	f	104	19.1	0.0019	1; 1; 0; 0; 0	0; 0; 0; 0; 0	0; 0; 0; 0; 0
St 38.8	m	139	30.0	0.0020	1; 1; 0; 0; 0	0; 0; 0; 0; 0	0; 0; 0; 0; 0
C 1. Rats refed one-half week after maintenance from three to twelve weeks of age							
St 12.53	m	85	48.5	0.0028	2; 2	0; 0	0; 0
St 10.25	f	87	40.5	0.0022	1; 2	0; 0	0; 0
C 2. Rats refed one week after maintenance from three to twelve weeks of age							
St 11.41	m	88	62.2	0.0031	12; 7; 10	0; 1; 0	0; 0; 0
St 10.27	f	89	55.0	0.0028	6	0	0
St 12.48	f	88	50.2	0.0028	7	0	0
St 11.45	f	88	67.6	0.0034	6	0	0
St 11.42	f	88	55.0	0.0031	2; 2	0; 0	0; 0

TABLE 5—Concluded

RAT NO.	SEX	AGE	GROSS BODY WEIGHT	HYPOPHYSIS WEIGHT	NUMBER OF MITOSES COUNTED IN ENTIRE CORONAL SECTIONS OF THE HYPOPHYSIS IN THE		
					Pars anterior	Pars intermedia	Pars nervosa
C 3. Rats refed two weeks after maintenance from three to twelve weeks of age							
		days	*grams*	*grams*			
St 12.51	f	95	77.2	0.0034	8	0	0
St 12.49	f	95	60.5	0.0027	5	0	0
St 11.40	f	95	84.5	0.0030	13; 8	0; 0	0; 0
St 11.43	f	95	79.0	0.0034	6	0	0
St 10.26	m	96	91.0	0.0033	5	0	0
C 4. Rats refed four weeks after maintenance from three to twelve weeks of age							
St 10.28	f	110	118.0	0.0068	1; 1	0; 1	0; 0
St 10.23	f	111	129.5	0.0094	5	0	0
St 12.47	f	109	106.5	0.0082	3	0	0
St 12.52	m	109	125.2	0.0056	5	0	0
St 11.46	f	109	118.0	0.0088	107; 112	0; 0	0; 0
C 5. Rat refed six months after maintenance from three to twenty weeks of age							
S 33.118	m	346	229.0	0.0094	0	0	0

a. During normal growth of the hypophysis

To determine the rate of mitosis in the normal gland at various ages, sections were examined from 14 individuals, varying from newborn to 225 days of age (tables 5 and 6). In the newborn, mitosis is very active throughout the gland, the average being 62 mitoses in each section for the pars anterior, 9 for the pars intermedia, and 7 for the pars nervosa. Considering the difference in the areas of the three lobes (fig. 1), it may be concluded that the actual number of mitoses for a given number of cells (the 'mitotic index' of Minot) would probably be similar in all three lobes, though somewhat less in the pars nervosa.

In the pars nervosa, mitoses soon disappear. From an average of 7 per section in the newborn, they decrease to an average of two-fifths (i.e. 2 mitoses found in 5 sections) at seven days. In later stages (twelve days and above) none was found in any case. Growth in the pars nervosa thereafter consists almost entirely in the accumulation of intercellular substances.

In the pars intermedia mitosis continues, but at a progressively decreasing rate. The decrease is even relatively greater than is apparent, on account of the progressive increase in the absolute size of the areas examined. The average of 9 mitoses per section in the newborn decreases to a little less than 1 at one week and three weeks, and to one-thirteenth at ten weeks.

TABLE 6

Summary of number of mitoses in sections of the hypophysis. Averages from table 5 (exceptional cases J 1.3 and St 11.46 being excluded)

AVERAGE AGE	NUMBER OF EACH SEX IN GROUP	CONDITION	AVERAGE NUMBER OF MITOSES PER SECTION IN		
			Pars anterior	Pars intermedia	Pars nervosa
days					
Newborn	1 m	Normal	62	9	7
7	1 f	Normal	18	$\frac{4}{5}$	$\frac{2}{5}$
21	1 m, 2 f	Normal	7	$\frac{22}{25}$	0
70	3 m, 1 f	Normal	2	$\frac{1}{13}$	0
145	1 m, 2 f	Normal	$\frac{5}{7}$	$\frac{1}{7}$	0
83	4 m, 4 f	Maintained from 3 weeks	$\frac{1}{4}$	0	0
86	1 m, 1 f	Refed $\frac{1}{2}$ week	$1\frac{3}{4}$	0	0
88	1 m, 4 f	Refed 1 week	7	$\frac{1}{8}$	0
95	1 m, 4 f	Refed 2 weeks	7	0	0
110	1 m, 3 f	Refed 4 weeks	3	$\frac{1}{7}$	0
346	1 m	Refed 6 months	0	0	0

It apparently increases somewhat in the older cases (even excluding J 1.3, which also shows an abnormally large number in the pars anterior), bringing the average up to one-seventh. This apparent increase is probably due to chance variation in the relatively small number of sections examined.

In the pars anterior, the rate of mitosis likewise decreases (tables 5 and 6), the average number being 62 at birth, 18 at one week, 7 at three weeks, 2 at ten weeks and five-sevenths in 3 older rats. From the last group is excluded one very exceptional case (J 1.3, 94 days) in which the number of mitoses varied from 8 to 12 per section. This represents an extreme individual variation. At the other extreme is St 47.5 (at 67 days) in which no mitoses were found in 3 entire sections. In this case the body weight was unusually high (196 grams), however, and growth had probably become very slow as the adult weight was approached. It is probable that occasional mitoses occur so long as growth continues.

b. Mitoses in young rats held at maintenance, and in adults with acute and chronic inanition

In the young rats held at maintenance from the age of three weeks to ten weeks or more, mitosis has nearly ceased (tables 5 and 6). No mitoses were observed in the partes intermedia and nervosa. In the pars anterior, however, mitoses still occur occasionally, the total number found in 24 sections being 6, or an average of one-fourth per section. It is of interest to note that occasional mitosis in the pars anterior persisted even in a rat held nearly at maintenance from three weeks to twenty weeks of age (St 38.8), and which was nearly dead from inanition when killed. In spite of this persistent mitosis, however, the pars anterior apparently suffers the greatest relative loss in volume, as previously shown.

No mitoses were observed in any of the older rats subjected to acute and chronic inanition. No systematic search was made for them over entire sections, however, so they are not included in the tables.

c. Mitoses in various stages of refeeding after maintenance

In the young rats refed after a period of maintenace (tables 5 C and 6), mitoses were never found in the pars nervosa. In the pars intermedia, in rats refed one to four weeks, they occur occasionally, about as often as in younger normal rats of corresponding weight. In the pars anterior, mitosis reappears promptly. In rats refed only one-half week, in 4 sections examined a total of 7 mitoses was observed (table 5 C 1), or an average of one and three-fourths per section. The rate increases to an average of about 7 mitoses per section in rats refed one week and two weeks, and about 3 in those refed four weeks.

No mitoses were found in one rat (S 33.118), which had been refed six months after maintenance from three to twenty weeks of age. This rat, however, had a body weight of 229 grams, and had nearly ceased to grow. In general, therefore, we may conclude that after one week or more of refeeding the rate of mitosis corresponds in general to that of normal younger rats of similar body weight.

In these refed rats, however, marked individual variations in the number of mitoses are found, both in different individuals and in different sections of the hypophysis of the same individual. These variations usually have no obvious cause. In one very exceptional case, however, an apparent cause was found. In rat St 11.46 (table 5 C 4), refed four weeks after maintenance from three to twelve weeks, an astonishing number of mitoses was found in the pars anterior, 107 being counted in one section and 112 in another. This enormous rate of mitosis was apparently somewhat evenly distributed throughout the lobe, though no actual counts were made in other sections. It seemed to involve all types of cells. No mitoses were found in the partes intermedia and nervosa. The apparent cause of this abnormal mitosis was found in a small inflammatory lesion near the center of the anterior (distal) lobe, which presented a circumscribed area filled with polymorphonuclear leucocytes. The stimulus from this lesion doubtless caused the proliferation of cells throughout the anterior lobe, while apparently not affecting the remainder of the gland.

CHANGES IN HISTOLOGICAL STRUCTURE

The normal histology of the hypophysis in the rat has been described briefly by Tilney ('11, '13) and by Stendell ('14). In the following description, only those features especially concerned in the changes during inanition will be considered.

a. Pars nervosa

In the newborn rat the pars nervosa in structure resembles a vascular mesenchyme, with numerous stellate or spindle-shaped (neuroglia) cells, whose cytoplasm fades off into a very fine intercellular network (neuroglia fibrils). The nuclei are rounded distinct, and moderately chromatic. In some cases they appear naked and nearly free from cytoplasm, which is the typical condition from the age of one week onward.

In later stages (3 weeks and above) the nuclei become progressively scattered, usually rounded or elliptical in form, and vesicular in appearance, with average diameter of about 6 μ (range 4 to 8 μ). The internuclear mass presents a fine plexus of (neuroglia) fibrillae, interspersed apparently with a granular matrix or ground substance. Among these granules there is found a variable number of spherical masses, sometimes exceeding the nuclei in size, and resembling 'colloid' in appearance. They doubtless correspond to the colloid masses described by Herring ('08) and Trautmann ('09) in the neural lobe of various animals.

The only change noted in the structure of the pars nervosa during inanition (either in young or adults) is a variable degree of hyperchromatism in the nuclei. In extreme cases, the nuclei rarely become somewhat irregular, shrunken and pycnotic. No definite change was observed in the internuclear mass, in the fibrillae, granules or 'colloid' balls.

b. Pars intermedia

The pars intermedia (fig. 1) forms an epithelial plate separating the pars nervosa from the residual lumen (hypophyseal cavity). This plate is only a few cells thick in the central region, but thicker peripherally. The cell boundaries are usually indistinct. From the age of three weeks onward the cells of the limiting layer (next to the lumen) are more or less flattened, and rarely present small ciliated areas.

In structure, the cells usually present cytoplasm filled with fine, pale violet granules, resembling those of the faintly basophilic cells of the pars anterior. The nuclei are round or oval, and moderately chromatic. Hyperchromatic and even pycnotic nuclei are occasionally found, but are rare in the normal animals. They are mentioned by Stendell ('14) as of uncertain significance.

During inanition, the pars intermedia presents a varied structure, but on the whole the changes are usually not very marked. These changes are somewhat similar in the young rats held at maintenance, and in the older rats subjected to acute and chronic inanition.

The cytoplasm during inanition as a rule does not appear less abundant than in the normal animal, except in certain atrophic areas of variable size and number. Here the cytoplasm may be scanty, and the nuclei closely packed. In structure, however, the cytoplasm usually appears altered, more rarefied and sparsely granular in appearance. Sometimes it assumes a markedly reticular or vacuolated appearance, but this is not constant. Around pycnotic nuclei, the cytoplasm usually assumes a more dense and homogeneous appearance, and stains more deeply basophilic.

The nuclei of the pars intermedia cells during inanition, according to the measurements above given (table 4) show a slight decrease in size, though frequently with but little evident change in structure. There is usually a definite tendency to hyperchromatism, however, less marked in some cases but distinct in others (especially when the inanition is extreme). In some cases, especially in the atrophic areas above referred to, the nuclei present variable degrees of pycnosis. Karyorrhexis and karyolysis are rare.

In this connection may be mentioned the 'colloid.' The colloid-like masses in the pars nervosa have already been considered. In the rat, colloid does not occur in the pars intermedia, but is usually found in the lumen of the hypophyseal cavity, at and after the age of three weeks. The amount is small, though somewhat variable. It presents marginal vacuoles resembling those in the colloid of the thyroid follicles. Trautmann ('09) mentions vacuoles in the colloid of the hypophysis of domestic animals, but considers them artefacts. If these vacuoles are interpreted as secretion phenomena, it is of interest to note that in the rat they appear more typically and frequently on the surface toward the pars intermedia, more rarely on the surface next to the pars anterior. No constant changes in the amount or structure of the colloid (either in the cavity or in the pars nervosa) were observed in the rats subjected to inanition.

c. Para anterior (distalis)

The parenchyma cells of the pars anterior present the well known types (figs. 2 and 4). These have been classified in various ways, the most useful for present purposes perhaps being that of Trautmann ('09). He recognizes the following classes of cells: (1) acidophilic (strongly or weakly); (2) basophilic (strongly or weakly); (3) chromophobic. The number, arrangement and structure of these cell-varieties differ considerably in different species; and, at least in the rat, in different individuals.

The chromophobes form an undifferentiated type of cell, relatively numerous in the newborn rat, but somewhat rare after the third week. The weakly basophilic type is usually the most numerous, the strongly basophilic rare. The acidophilic (eosinophilic) type is evident in the newborn and at three weeks, though much better differentiated later. It usually forms at least one-third of the total number of cells. The weakly acidophilic are more numerous than the strongly acidophilic, though it would be hard to draw the line between them. In fact, there appear to be numerous transition forms between all varieties (especially the chromophobic and weakly basophilic).

Tilney ('11) describes the basophiles as occupying the periphery, and the acidophiles the central region. I do not find any constant arrangement of this sort. The acidophiles are usually scattered throughout the lobe, either singly or in small groups, interspersed with basophiles. Sometimes, however, either basophiles or acidophiles may predominate in the periphery.

In the normal gland, the cytoplasm of the chromophobic cells is very small in amount, sparsely granular, and faintly staining (fig. 2). In the basophilic and acidophilic types, the cytoplasm is more abundant and usually finely (sometimes coarsely) granular in appearance. In the strongly chromophilic cells, however, the cytoplasm tends to become more homogeneous in appearance. In the eosinophiles, at least, this is apparently because the stain affects the intergranular substance as well as the granules of the cytoplasm (figs. 2 and 4). Sometimes the cytoplasm presents a finely vacuolated appearance. Various developmental stages of the 'ring-cell' type described by Addison ('16) are occasionally met in rats of ten weeks or more. The cell-boundaries of the acidophiles (eosinophiles) are usually distinct, while those of the basophiles and chromophobes are indistinct. The various types of cells in the hypophysis of the normal rat are well shown by colored figures in the second volume of Biedl ('13).

The nuclei of the chromophobic and weakly chromophilic cells are spherical or ellipsoidal in form and similar in structure, presenting a distinct nuclear membrane and an indistinct, irregular nuclear reticulum with one or more distinct karyosomes (figs. 2 and 4). The nuclei are somewhat vesicular in type and only moderately chromatic. To a certain extent, the amount of chromatin appears to vary inversely with the age, since the nuclei stain more deeply in the younger stages (up to three weeks) than in the older (ten weeks or more).

The nuclei of the eosinophilic cells also show a tendency to more highly chromatic (deeply staining) condition, which is especially marked in the strongly eosinophilic (as noted by Stendell '14), as well as in the rarer strongly basophilic cells. In the strongly eosinophilic cells, it was frequently noted that the nuclear matrix (karyolymph) becomes acidophilic, staining red like the cytoplasm. It may likewise appear purplish in the strongly basophilic type.

In a few of the strongly chromophilic cells the nuclei may in extreme cases present even a pycnotic condition. This tendency to pycnosis is usually more evident toward the periphery, near the surface of the gland and usually involves more or less atrophy of the cytoplasm. In addition to these apparently normal pycnoses, others may be observed in the region of injuries produced during removal of the gland. This was found also in the thyroid gland (Jackson '16). Pycnotic nuclei, possibly degenerative in character, were observed by Schönemann ('92) in the pars anterior of the human hypophysis, and by Stendell ('14) in the rat.

During inanition, the changes in the parenchyma cells of the pars anterior are similar in the young rats held at maintenance, and in older rats subjected to acute and chronic inanition. As might be expected, the changes are usually more marked in cases where the inanition is more protracted or severe. The changes vary greatly even in different parts of the same individual, so that great care is necessary in drawing generalized conclusions. Some areas may even remain nearly normal in appearance, while others show extreme changes of atrophy and degeneration. In general, such changes are usually found more marked and extensive in the peripheral portions of the gland. The surrounding pressure might render the surface layers of an organ somewhat more prone to atrophy, but it is difficult to understand why this should be so pronounced in the case of the hypophysis.

As already shown, there is during inanition usually a marked loss in the volume (both relative and absolute) of the cytoplasm. Even in areas retaining a considerable amount of cytoplasm, its structure frequently becomes coarsely vacuolated. In other cases (especially during maintenance and chronic inanition) these vacuoles apparently coalesce to form a watery intercellular substance, leaving the nearly naked nuclei surrounded by a thin layer of cytoplasm (fig. 3). On account of such changes, together with the hyperemia characteristic of inanition, it is evident that the loss in protoplasmic substance may be (and usually is) proportionately much greater than the loss in gross weight of the gland.

The cytoplasm also tends in general to lose its specific staining reactions. The strongly chromophilic types stain more faintly, and the weakly chromophilic become chromophobic. In extreme cases, and in areas of marked atrophy, all cells may become chromophobic, with no trace of acidophilic or basophilic reactions. For the most part, however, the cytoplasm does not reach this stage, but becomes more or less sparsely granular, with a corresponding diminishing intensity of the specific stain. In some regions (especially in the central portion of the gland) the staining reactions may be preserved much better than elsewhere.

Corresponding to the cytoplasmic atrophy, there are also changes in the nucleus. That there is loss in volume (though less than in the cytoplasm) in the nucleus has already been shown by measurements. In structure, some areas may show relatively little nuclear change, but in the great majority of cases there is a very decided hyperchromatism (figs. 3 and 5). This is best marked in the peripheral atrophic regions above referred to, where all the nuclei may present the typical pycnotic condition, shrunken, deeply staining and homogeneous. Or intermediate stages may occur, in which the nuclei are larger, with the chromatin dissolved into a pale-blue homogeneous matrix which obscures the nuclear network. Karyorrhexis and karyolysis also occur, but are comparatively rare.

In cases or areas where the atrophic changes are not so far advanced, it appears that the pycnosis first becomes evident in the nuclei of the eosinophiles. This might be expected since, as above stated, hyperchromatism and even occasional pycnosis occur in the strongly chromophilic cells of the normal gland. In some cases during inanition it appears also that the acidophilic reaction of the karyolymph may persist longer in the nucleus than in the cytoplasm of the eosinophiles. This may be due to physical rather than chemical conditions, however.

d. Changes during refeeding after maintenance

Associated with the mitoses already noted in the hypophysis upon refeeding after maintenance, changes take place in the cells leading to a restoration of the normal structure, at least in the greater part of the gland.

After one-half week of refeeding, although the gland has increased appreciably in weight, and mitosis has begun in the pars anterior, the cell structure typical for inanition still persists to a very large degree. The only changes noted are a lessening of the hyperemia in the pars anterior, and some increase in the amount of cytoplasm, with perhaps a slight decrease in the hyperchromatism of the nuclei in a few places. In general, however, it would be difficult to distinguish this stage from that at the end of the maintenance period.

After one week of refeeding, however, some areas (especially in the partes intermedia and nervosa) have become nearly normal in histological structure. In the pars anterior, these areas are more frequently found in the central region, and their extent varies considerably in different individuals. In general, however, the structure characteristic of inanition still prevails over the greater part of the gland.

After two weeks of refeeding, the normal structure becomes progressively more evident, and usually prevails over the greater part of the gland. The cytoplasm and nuclei become nearly normal in size and structure. Both pars intermedia and pars anterior still retain atrophic areas, however, especially in the periphery of the latter.

Even after four weeks or more of refeeding, although the gland in general has usually become nearly normal in structure, some more or less atrophic areas may still persist. These are much more frequent and extensive than the similar areas previously mentioned as occasionally found in the normal gland, which usually involve only single cells or small groups.

The recovery of the cells upon refeeding apparently depends upon the extent of degeneration (especially of the nucleus) involved during the inanition period. It is probable that nuclei in the extreme stages of pycnosis are beyond the possibility of repair, although they may persist in this condition for a long time before disintegration and removal.

DISCUSSION AND CONCLUSIONS

The effects of inanition upon the hypophysis were studied by Guerrini ('04). He found in the hypophysis (anterior lobe) of 4 dogs, 4 rabbits and 4 pigeons, during the first third of the acute inanition period, a slight increase of secretory activity in the cells, as indicated by a more intense reaction to Galeotti's stain. In the remaining period of acute inanition, however, he found a progressive decrease in staining capacity (granules and plasmosomes) with vacuolization of the cytoplasm. The final appearances are described as follows:

> Negli animali morti di fame, uso l'espressione nel senso il più lato, le cellule sono tutte, o presso che tutte, ridotte come in vesicole, quali più e quali meno gonfie, con nucleo, anch'esso, un po' vuoto e rigonfio e il protoplasma ridotto ad un velo, interrotto qua e là di qualche vacuolo e con appena una traccia di granuli o di plasmosomi.

In several dogs and rabbits (both young and adult) subjected to chronic inanition, however, Guerrini found no apparent change in the secretion (specific staining reactions) of the hypophysis cells. Unfortunately no details are given as to the exact character and extent of the chronic inanition. Possibly his negative results may be due to the comparative mildness of the inanition. His findings in the later stages of acute inanition are in general agreement with mine, excepting that he describes the nuclei as vesicular rather than pycnotic in structure.

It is a noteworthy fact that most of the changes above described in the cells of the hypophysis—arrested mitosis (resumed on refeeding), shrinkage in cell volume, nuclear pycnosis and loss of specific cytoplasmic staining reactions—are strikingly similar to those found in the hypophysis of the hibernating marmot (woodchuck) by Gemelli ('06) and by Cushing and Goetsch ('15). Mann ('16), however, points out that if such changes are the cause of hibernation, they should appear well-marked at the beginning of hibernation, since later they might be merely a result of the long continued torpid state. He finds these changes inconstant in the hibernating gopher (Spermophilus). In view of the striking similarity of the cell changes in the two conditions (hibernation and inanition), it seems highly probable that the changes described in the hypophysis during hibernation are simply the effects of the chronic inanition involved.

Since the rapid growth of the body in young rats upon refeeding after a period of maintenance precedes the reëstablishment of the specific cell-granules (acidophile or basophile) in the hypophysis, it is evident that their function can not be the cause of the body growth. It is, however, at least theoretically, possible that the absence of cell-granules might indicate a hyperfunctional condition, in which the granules are absorbed too rapidly to allow them to form the normal accumulation in the cytoplasm. Moreover it should be remembered that the granules are rarely absent altogether. In most cases they are found even in extreme inanition, though in scattered areas and more or less reduced in amount. It is likewise evident that the 'colloid' can scarcely be considered as the functional cause of growth, for, although it persists apparently unaffected by inanition, it is normally absent in the very young animals, in which the growth rate is more rapid. It may indeed be recalled that the most rapid growth of the body in all cases occurs in the early embryonic period, preceding any differentiation whatever in the hypophysis.

So far as the hypophysis is concerned, therefore, no evidence appears in favor of the suggestion by Osborne and Mendel ('16) that the accelerated growth following periods of suppression may be due to specific histological changes in the ductless glands. Likewise no such changes were found in the thyroid and parathyroid glands (Jackson '16). There is, however, some evidence in favor of the view that the rapid growth upon refeeding is due to the embryonic type of structure produced by the inanition in the cells of the body in general (Stewart '16). The nuclei become relatively larger and richer in chromatin, the cytoplasm small in amount and undifferentiated in structure. According to the theory of Minot ('07), these are the characteristics upon which the more rapid growth of embryonic cells depend.

While the cell changes produced by inanition may in general facilitate rapid growth upon refeeding, when pushed to the extreme (as above shown) the cells degenerate to such an extent that recovery appears impossible. This is in agreement with the generally accepted doctrine that severe inanition in young animals may produce permanent stunting of body growth; although Osborne and Mendel ('16) obtained no permanent stunting by long periods of growth suppression in the albino rat.

SUMMARY

1. During normal postnatal growth there is considerable individual variation in the relative volumes of the three parts (lobes) of the hypophysis; but in general in the older rats the pars anterior (distalis) becomes relatively larger, and the pars nervosa correspondingly smaller, the pars intermedia remaining about the same in relative (percentage) volume.

2. The relatively larger hypophysis of the female rats is due chiefly (if not entirely) to a larger anterior lobe.

3. During inanition, the volume-changes in the lobes are variable. In young rats held at maintenance (constant body weight), the pars anterior is somewhat reduced, the intermedia and nervosa correspondingly larger. In chronic (adult) inanition the partes anterior and intermedia appear reduced, the nervosa increased. In acute (adult) inanition, the pars anterior appears relatively increased, intermedia decreased, and nervosa unchanged in relative volume.

4. In young rats refed one-half week, one week, two weeks and four weeks after maintenance, there is some variability, but in general a gradual return to the normal proportions in the lobes of the hypophysis. After a prolonged period of maintenance, however, the relative volume of the lobes may remain permanently abnormal.

5. In the pars anterior of the normal newborn rat, the vessels and associated stroma form 6.7 per cent by volume, increasing to 9.6 per cent at three weeks, and to 10.6 per cent at ten weeks (adult condition). In young animals held at maintenance, the volume of the vascular stroma usually increases to about 13 per cent, and in acute or chronic inanition of adults to about 17 per cent. The parenchyma is, of course, correspondingly reduced in relative volume.

6. In the parenchyma of the pars anterior the nuclei form about 34 per cent of the total cell volume in the newborn, decreasing to about 24 per cent at three weeks and to 20 per cent at ten weeks (adult relation). The cytoplasm increases correspondingly in relative volume. During inanition, the loss is usually greater in the cytoplasm, the nuclei thereby increasing

to 26–28 per cent of the cell volume in the young held at maintenance, and to 23–26 per cent in adults with chronic or acute inanition.

7. According to data obtained by the volumetric method, the (calculated) average diameter of the parenchyma cells of the anterior lobe increases from 10.1 μ in the normal newborn to 11.9 μ at three weeks and 13.6 μ at ten weeks (adult condition). In young rats held at maintenance, the average cell diameter is reduced to 9.7–10.2 μ; in starved adults to 10.8–11.0 μ. The nuclear diameter averages 5.9 μ in the normal newborn, 5.8 μ at three weeks, and 6.0 μ at ten weeks. In the young rats at maintenance the nuclear diameter is reduced to 4.9–5.3 μ; in starved adults to 5.3–5.5 μ. Direct measurements by another method (with filar micrometer) gave similar results for the nuclear diameters, including also those of the pars intermedia.

8. The number of mitoses in an entire section of the gland is quite variable. Amitosis was never observed. In the normal newborn pars nervosa, the average number of mitoses is 7 per section; at seven days they are rare, and none occur later. In the pars intermedia, the average number decreases from 9 per section in the newborn to about 1 at three weeks; at ten weeks and later they are rare. In the normal pars anterior the rate likewise decreases, being about 62 at birth, 18 at one week, 7 at three weeks, 2 at ten weeks, and rare in adults.

9. In young rats held at maintenance from three to ten weeks of age, mitosis has nearly ceased. No mitoses were found in the partes nervosa and intermedia, although in the pars anterior they still occur occasionally, even in rats nearly dead from inanition. No mitoses were observed in the starved adults.

10. In the young rats refed after the maintenance period, mitoses reappear promptly in the pars anterior, the average number per section being about 2 after one-half week of refeeding, 7 after one week to two weeks, decreasing to an average of 3 after four weeks of refeeding. Mitoses were observed but rarely in the pars intermedia, and never in the pars nervosa. The rate of mitosis in the hypophysis of the refed rats therefore

corresponds roughly to that in younger normal rats of similar body weight.

11. In cell structure, the only change noted in the pars nervosa during inanition is a variable degree of hyperchromatism in the nuclei, which rarely may become shrunken and pycnotic. In the pars intermedia, most of the cells usually suffer relatively little change during inanition. The nuclei have a variable tendency to hyperchromatism, occasionally becoming pycnotic, especially in certain atrophic areas. The cytoplasm tends to lose its granular structure, becoming more homogeneous and often finely vacuolated in appearance. Around pycnotic nuclei it is usually more strongly basophilic, and is much reduced in volume in the atrophic areas above mentioned.

12. The colloid which occurs normally in the pars nervosa and in the hypophyseal cavity (residual lumen) appears unaffected by inanition.

13. In the pars anterior, the changes during inanition are quite variable. Some areas may remain nearly normal, while others, even in the same gland, show extreme changes of atrophy and degeneration. The cytoplasm is usually reduced in volume (as above shown) and is frequently much vacuolated. The structure becomes sparsely granular and there is a marked tendency to loss of the specific staining reactions, so that the strongly chromophilic cells become weakly chromophilic or even chromophobic. The nuclear changes are likewise variable, but there is a very general tendency to hyperchromatism, often reaching a definite pycnosis. Karyorrhexis and karyolysis are rare.

14. Upon refeeding one-half week after the maintenance period (three to twelve weeks of age), the hypophysis still retains the typical inanition structure, although mitosis and growth have begun. After one week of refeeding, some areas have become nearly normal, and after two weeks the normal structure preponderates. After four weeks, the greater part of the hypophysis appears nearly normal, although atrophic areas may persist for indefinite periods. Recovery is improbable in cells whose nuclei have reached advanced pycnosis.

15. The changes described in the hypophysis during hibernation are probably inanition effects. The rapid growth of the body following periods of inanition may be due to the embryonic condition to which the cells are reduced; but there is no evidence that such growth is due to any specific histological changes in the hypophysis or other ductless glands.

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