J. Embryol. exp. Morph., Vol. 12, Part 1, pp. 113–121, March 1964
Printed in Great Britain

Dry mass, lipid content and protein content of the intact and zona-free mouse ovum

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INTRODUCTION

MAMMALIAN ova have not been studied as thoroughly as the eggs of lower vertebrates. Increasing interest in the physiology and biochemistry of mammalian embryogenesis in recent years has been met with little quantitative information. Up to now there have been no direct chemical analyses of mammalian ova published. Reported here are the dry mass and protein and lipid contents of the single cell fertilized mouse ovum. The mouse was chosen because of its frequent use as an experimental animal for the study of developmental genetics and because large numbers of mouse ova can be obtained with relative ease. Single cell stage ova from mated females were chosen as the starting point from which to follow these parameters through cleavage and blastocyst formation, although it must be realized that a proportion of such ova would not be fertilized.

Besides being of general interest to embryologists, information such as this is necessary for the interpretation of respiratory studies (Boell & Nicholas, 1948; Fridhandler, Hafez & Pincus, 1956, 1957; Fridhandler, 1961) and for the comparison of respiratory rates of ova with the respiratory rates of other tissues. When enzymes assays are undertaken it will be necessary to know the protein content in order to quantify enzyme specific activity. The techniques demonstrated in this study can be applied to the study of fertility and sterility and to the investigation of the effects of maternal nutritional status and genetic background on development.

MATERIALS AND METHODS

Female 129/J mice, 3 weeks to 7 months of age, were induced to ovulate in the following way. Each mouse received 4 units of pregnant mares' serum (PMS—Ayerst) by intraperitoneal injection at 4 p.m. one day. At 1 p.m. on the second day afterward each received an intraperitoneal injection of 2 or 2·5 units of human

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chorionic gonadotropin (Antuitrin-S—Parke, Davis & Co.) and was placed in a cage with a fertile male. Mating occurred that evening. Using a similar dosage schedule, Runner (1950) reported that ovulation occurred 12 to 14 hr. after the second injection. The next morning the females were killed by cervical dislocation and their oviducts excised. The dilated ampulla, which contained the eggs, was dissected from the rest of the oviduct in a bath of saline; the ova were extruded from the ampulla by gentle pressure applied with a small knife blade. As many as ninety eggs were recovered from a single mouse. Ova obtained in this manner have been shown to be at least as viable as those recovered following spontaneous ovulation (McLaren & Michie, 1956; Gates, 1956).

About a dozen mice were processed in a day. Usually the ova recovered from all were pooled to facilitate handling. Braking pipettes were used to transfer the eggs from bath to bath. The basic medium used was 0·85 per cent. sodium chloride solution. The saline was buffered to pH 7·4 with phosphate (0·014M) when zona-free ova were handled, as they tended to lyse in unbuffered saline. Both buffered and unbuffered saline was used for eggs with zona intact; the data obtained from intact ova washed in buffered or unbuffered saline were not significantly different.

All ova were washed 12 to 66 (usually 20 to 40) min. in 0·01 per cent. hyaluronidase (Nutritional Biochemicals Corp.) in saline to remove adherent corona radiata cells, then transferred to a bath of saline in a depression slide. Those ova in which the zona was to be kept intact were washed an additional two to four times in saline. The zona pellucida was removed from other ova by digestion for 8 to 13 min. in 0·5 per cent. *Streptomyces griseus* protease (Pronase–Calbiochem) in Hanks' balanced salt solution at room temperature. Mintz (1962) has transplanted similarly treated ova into uterine foster-mothers and found these eggs to be viable. After removal of the zona the eggs were washed five times in buffered saline.

For protein analysis, groups of ova were taken from the last saline bath and frozen in unbuffered saline in test tubes, then dried *in vacuo*. The samples were assayed for total protein by the method of Lowry, Rosebrough, Farr & Randall (1951) against a standard of nitrogen-assayed human serum protein obtained from Dr Helen Burch. Blanks of saline stock and wash solutions were also analyzed to correct for the possible presence of hyaluronidase, protease or other contaminants.

Ova to be weighed were taken from the last saline bath, washed three times in 0·78 per cent. ammonium chloride solution to dilute out the sodium chloride, frozen and dried *in vacuo*. The ammonium chloride crystals remaining after drying were removed by sublimation on heating with infra-red radiation for 3 to 6 hr. The dry ova were weighed individually on a quartz fiber fishpole balance (Lowry, 1953), after which their lipid was extracted with a mixture of 2 parts chloroform and 1 part methanol followed by *n*-hexane. The ova were dried in a desiccator and reweighed. The difference between total dry mass and lipid-free dry mass was taken as the lipid content.

DATA

Both the extraction of lipid and the removal of the zona pellucida produced statistically significant changes in dry mass (see Table 1). Subject to a reservation to be discussed later, the difference in total dry mass of intact and zona-free ova is

TABLE 1

Dry mass of intact and zona-free ova before and after lipid extraction

	Intact ova		*Zona-free ova*	
	Mean ± std. deviation (mγ)	*No. of ova*	*Mean ± std. deviation (mγ)*	*No. of ova*
Total dry mass	31·97 ± 5·87	77	25·95 ± 3·28	38
Lipid-free dry mass	28·14 ± 5·45	62	22·70 ± 3·54	31

2 × 2 factorial analysis of variance.
Lipid extraction, $p < 0{\cdot}02$; Zona removal, $p < 0{\cdot}02$; Interaction, $p > 0{\cdot}10$.

TABLE 2

Protein content of intact and zona-free ova

	Intact ova		*Zona-free ova*	
	Mean ± std. deviation (mγ)	*No. of tubes*	*Mean ± std. deviation (mγ)*	*No. of tubes*
Protein per ovum	20·2 ± 3·8	67	18·4 ± 3·8	36

t-test for unpaired data. $p < 0{\cdot}05$.

TABLE 3

Dry mass, lipid content, and protein content of intact and zona-free ova and zona pellucida

	Intact ova	*Zona-free ova*	*Zona pellucida*
Total dry mass (mγ)	31·97	25·95	6·02
Lipid content			
(mγ)	3·83	3·25	0·58*
(per cent. of dry mass)	12·0	12·5	9·6*
Protein content			
(mγ)	20·2	18·4	1·8
(per cent. of dry mass)	63	71	30

* Not statistically significant.

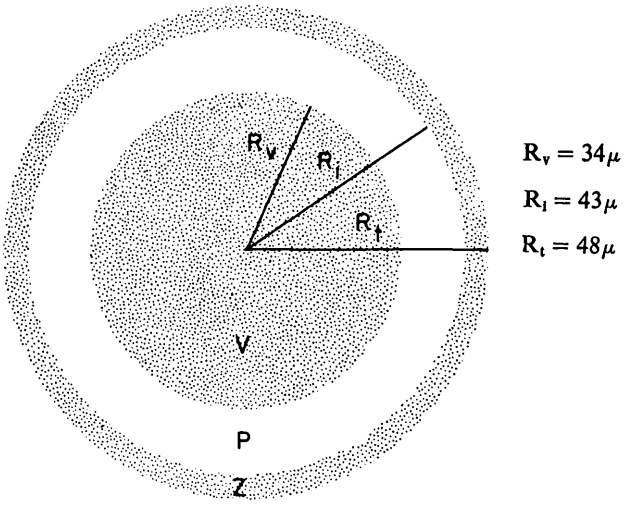
the total dry mass of the zona pellucida. This was 6·02 mμg., 19 per cent. of the dry mass of the intact ovum. The lipid content of the intact ovum was 3·83 mμg., 12·0 per cent. of the total dry mass; the lipid content of the zona-free ovum was 3·25 mμg., 12·5 per cent. of the total dry mass. The difference between total and lipid-free dry masses of the zona pellucida was 0·58 mμg., 9·6 per cent. of the

total dry mass of the zona; this difference, however, was not statistically significant.

The protein analyses are summarized in Table 2. The mean protein content of the intact ovum was 20·2 mμg., 63 per cent. of total dry mass. The mean protein for zona-free ova was 18·4 mμg., 71 per cent. of total dry mass. The difference between these means was statistically significant, giving a protein content for the zona of 1·8 mμg., 30 per cent. of total zona dry mass. Table 3 is a summary of the data.

DISCUSSION

Text-fig. 1 is a diagram of a typical ovum. In the single cell stage the ovum is very nearly a sphere. On that basis the volume of the vitellus can be calculated as $1{\cdot}65 \times 10^5\ \mu^3$. Likewise, the volume of the zona, which is the difference between



TEXT-FIG. 1. Diagram of the intact ovum. V, vitellus; P, perivitelline space; Z, zona pellucida; R_v, radius of vitellus; R_i, inner radius of zona pellucida; R_t, outer radius of zona pellucida.

the volumes of two spheres, is $1{\cdot}30 = 10^5\ \mu^3$. In spite of its thinness, the zona is almost as large as the vitellus. Lewis & Wright (1935) found the zona of other strains of mice to be even larger than the vitellus. Because of the large volume of the zona, analyses of intact ova may not accurately determine the composition of the egg cell cytoplasm. This is why it was necessary to analyze ova from which the zona had been removed; zona-free eggs are almost entirely cytoplasm.

Weiss (1958) observed that Sarcoma 37 ascites cells incubated in trypsin lost 20 per cent. of their dry mass without any change in volume or viability. The question might be raised as to whether or not this phenomenon occurred when the ova were incubated in *Streptomyces griseus* protease. The decrease in dry mass noted for the ova was 19 per cent. However, the protease digestion also resulted in loss of the zona pellucida, which made up half the volume of the ovum;

furthermore, only 30 per cent. of the dry mass lost was protein. If an effect such as Weiss noted were operating here, a much greater decrease in dry mass would have been expected. It is unlikely, therefore, that such a phenomenon occurred to any appreciable extent in this case.

The specific gravity of the sea-urchin egg, which, like the mammalian ovum, is microlecithal, is 1·08 (Harvey, 1932). Using this value, the wet mass of the mouse ovum may be estimated as 178 mμg. for the vitellus and 140 mμg. for the zona pellucida. If these estimates be correct, then the cytoplasm is 15 per cent. solid matter and 85 per cent. water, while the zona is 4 per cent. solid matter and 96 per cent. water. This finding is supported by Harter's (1948) observation that after freeze-drying the zona has a highly reticulated appearance which he interpreted as indicating a relatively high concentration of water in the zona.

The many histochemical and related studies of the zona pellucida have pointed out a number of species differences, but it is generally agreed that protein and mucopolysaccharide are the chief components. Protein has been demonstrated by histochemical staining by specific reactions (Braden, 1952) and by digestion of the zona with proteolytic enzymes (Braden, 1952; Smithberg, 1953; Chang & Hunt, 1956; Mintz, 1962). Protein denaturing agents, such as urea (Braden 1952) and trichloroacetic acid, will remove the zona from the eggs of rats and mice, respectively; urea does not remove the rabbit zona, however. Braden (1952) found that oxidizing agents such as hydrogen peroxide removed the zona from rat and rabbit eggs. The nature of the protein is unknown. The use of *Streptomyces griseus* protease, which completely hydrolyzes all proteins to amino acids without specificity for certain peptide bonds (Nomoto, Narahashi & Murakami, 1960*a*, *b*), does not add any information about the protein of the zona.

The zona of the rat and rabbit (Deane, 1952; Braden, 1952) is deeply stained with the periodic acid-Schiff (PAS) technique, indicating the presence of mucoids or glycoproteins (Stary, 1959). Metachromatic staining of the zona has been demonstrated in the cat (Konecny, 1959), the rabbit (Braden, 1952) and the sow (Wislocki, Bunting & Dempsey, 1947), but was not found in the rat (Wislocki *et al.*, 1947; Deane, 1952; Braden, 1952). In the case of the cat, metachromasia is not found after pre-treatment of the section with hyaluronidase (Konecny, 1959), indicating that the mucopolysaccharide component is largely hyaluronic acid (Wislocki *et al.*, 1947). The metachromasia of the sow zona is not destroyed by hyaluronidase; this fact and the demonstration of a saliva-resistant Bauer reaction by Wislocki *et al.* (1947) suggest a preponderance of sulfated mucopolysaccharides. The absence of metachromatic staining from the rat zona could indicate a preponderance of neutral polysaccharides, but the presence of highly polymerized hyaluronic acid could produce the same effect (Stary, 1959). Furthermore, Braden (1952) showed that the acidic groups in the rat zona and hyaluronic acid had similar dissociation curves. Attempts by the authors to digest the mouse zona with hyaluronidase failed at neutral pH but were successful

at pH 4·6; this latter concentration of hydrogen ions is sufficient in itself to digest the mouse zona (Hall, 1935), and it was not clear whether the presence of the enzyme facilitated the digestion.

None of the histochemical studies indicates the relative amounts of protein and carbohydrate in the zona pellucida. Glycoproteins from various biological sources have been studied and found to vary in composition from almost all protein to almost all carbohydrate (Stary, 1959). This study found the dry mass of the mouse zona to be 30 per cent. protein. Since no statistically significant amount of lipid could be demonstrated, the remaining 70 per cent. of the dry mass of the zona is presumably carbohydrate. The virtual absence of demonstrable lipid confirms work by Braden (1952) ,who was unable to demonstrate sudanophilia in the zona of the rat or the rabbit. Konecny (1959), however, interpreted the staining of the cat zona by Sudan B as indicating the presence of a lipoprotein component.

Mammalian ova are microlecithal, but the amount of yolk present varies from species to species. The ova of rats and mice are entirely free from visible cytoplasmic inclusions by light microscopy (Corner, 1928). The absence of large yolk inclusion bodies has been confirmed by the electron microscopic studies of the rat ovum by Sotelo & Porter (1959). The amount of yolk present may be estimated quantitatively from the lipid content of the egg. Sixty-one per cent. of dried hen-egg yolk is lipid (Burton, 1958). If mammalian yolk in general or mouse yolk in particular be of similar composition, and if all the measurable lipid be assumed part of the yolk, then yolk would contribute no more than 20·5 per cent. of the dry mass of the zona-free mouse ovum. The actual percentage must be somewhat less, because at least part of the measurable lipid is contributed by the structural material of the cell organelles. According to Giese (1957) and Sponsler & Bath (1942), lipid contributes 10 to 13 per cent. of the dry mass of protoplasm from various sources. The fact that the lipid content of the mouse ovum does not exceed that of other protoplasm indicates that the major portion of the egg lipid is non-vitelline and that the actual yolk concentration is only a small fraction of the estimated maximum.

16·5 per cent. of the dry mass of the zona-free ovum was neither protein nor lipid; probably most of this was carbohydrate. Three different histochemical techniques have failed to show any large glycogen stores in the rat ovum (Wislocki *et al.*, 1947; Harter, 1948; Deane, 1952). The mammalian egg is said to lack large stores of nucleic acid (Austin, 1961), and Alfert (1950) found that ribonucleic acid did not become abundant in the cytoplasm of the mouse ovum until the time of implantation.

A comparison of the zona-free mouse ovum with protoplasm from other sources appears in Table 4.

Weighing the ova individually provided a measure of the variability in mass. Intact ova were significantly more variable than zona-free ova when compared by Bartlett's chi square test for homogeneity of variance. This indicates that the zonae are more variable in mass than the vitelli.

TABLE 4

Comparison of the zona-free mouse ovum with protoplasm from other sources

Author	*Material*	*Composition of dry mass*		*Dry mass as percentage of wet mass*
		Protein (%)	*Lipid* (%)	
Giese (1957)	Cell protoplasm	67–80	12–13	15–25
Sponsler & Bath (1942)	Cell protoplasm	67–80	10–13	10–15
Ephrussi (1933)	Sea urchin egg	66	21	23
This study	Mouse ovum	71	12·5	15*

* Estimated.

SUMMARY

1. Intact and zona-free single-celled mouse ova were analyzed for total protein and were dried and weighed on a quartz fiber fishpole balance before and after lipid extraction.

2. The zona pellucida was found to contribute 19 per cent. of the dry mass of the intact ovum. It was estimated to be 96 per cent. water and 4 per cent. solid matter, of which 30 per cent. was protein. No statistically significant amount of lipid was found.

3. The cytoplasm was estimated to be 85 per cent. water and 15 per cent. solid matter, of which 12·5 per cent. was lipid and 71 per cent. protein. The lipid content was no greater than that of protoplasm from other sources, indicating the presence of only a small amount of yolk.

4. The cytoplasm was found to be less variable in mass than the zona pellucida.

RÉSUMÉ

Poids sec, contenu en lipides et en protéines de l'oeuf de Souris tant intact que libéré ce sa zone pellucide

1. Des oeufs de Souris indivis, soit intacts soit libérés de leur zone pellucide ont été l'objet d'un dosage de leurs protéines; ils ont d'autre part été soumis, après séchage, à une pesée sur balance à fibre de quartz, montée 'en canne à pêche,' avant et après leur extraction lipidique.

2. Il a été trouvé que la zone pellucide intervient pour 19% dans le poids sec de l'oeuf intact. On a estimé qu'elle contient 96% d'eau et 4% de matière solide, dont 30% de protéine. Aucune valeur statistiquement significative n'a été obtenue en ce qui concerne les lipides.

3. Le cytoplasme a été estimé contenir 85% d'eau et 15% de substance solide, dont 12,5% de lipide et 71% de protéine' Le contenu lipidique n'est pas plus élevé que celui de protoplasme d'autre origine, ce qui indique que l'oeuf contient seulement une petite quantité de vitellus.

4. La masse du cytoplasme s'est trouvée moins variable que celle de la zone pellucide.

ACKNOWLEDGEMENTS

This work was supported in part by U.S. Public Health Service Grant RG 7730 and Training Grant S Tl GM-881-02. The authors are indebted to Professor Jack Davies for his generous assistance.

REFERENCES

ALFERT, M. (1950). A cytochemical study of oogenesis and cleavage in the mouse. *J. cell. comp. Physiol.* **36**, 381–409.

AUSTIN, C. R. (1961). *The Mammalian Egg.* Oxford: Blackwell Scientific Publications.

BOELL, E. & NICHOLAS, J. (1948). Respiratory metabolism of the mammalian egg. *J. exp. Zool.* **109**, 267–81.

BRADEN, A. (1952). Properties of the membranes of rat and rabbit eggs. *Aust. J. sci. Res.* (b), **5**, 460–71.

BURTON, B. (1958). *Nutritional Data.* Pittsburgh: H. J. Heinz Co.

CHANG, M. & HUNT, D. (1956). Effects of proteolytic enzymes on the zona pellucida of fertilized and unfertilized mammalian eggs. *Exp. Cell Res.* **11**, 497–9.

CORNER, G. (1928). Cytology of the ovum, ovary and fallopian tube. In *Special Cytology* (ed. E. V. Cowdry). New York: Paul B. Hoeber, Inc.

DEANE, H. (1952). Histochemical observations on the ovary and oviduct of the albino rat during the estrous cycle. *Amer. J. Anat.* **91**, 363–413.

EPHRUSSI, B. (1933). Contribution a l'analyse des premiers stades du développement de l'oeuf. Action de la température. *Arch. Biol., Paris et Liège*, **44**, 1–147.

FRIDHANDLER, L. (1961). Pathways of glucose metabolism in fertilized rabbit ova at various pre-implantation stages. *Exp. Cell Res.* **22**, 303–16.

FRIDHANDLER, L., HAFEZ, E. & PINCUS, G. (1956). Respiratory metabolism of mammalian eggs. *Proc. Soc. exp. Biol., N. Y.* **92**, 127–9.

FRIDHANDLER, L., HAFEZ, E. & PINCUS, G. (1957). Developmental changes in the respiratory activity of rabbit ova. *Exp. Cell Res.* **13**, 132–9.

GATES, A. (1956). Viability and developmental capacity of eggs from immature mice treated with gonadatrophins. *Nature, Lond.* **177**, 754–5.

GIESE, A. (1957). *Cell Physiology.* Philadelphia: W. S. Saunders Co.

HALL, B. (1935). The reactions of rat and mouse eggs to the hydrogen ion. *Proc. Soc. exp. Biol., N. Y.*, **32**, 747–8.

HARTER, B. (1948). Glycogen and carbohydrate-protein complexes in the ovary of the white rat during the oestrous cycle. *Anat. Rec.* **102**, 349–67.

HARVEY, E. (1932). Physical and chemical constants of the egg of the sea urchin, *Arbacia punctulata. Biol. Bull. Wood's Hole*, **62**, 141–54.

KONECNY, M. (1959). Etude histochimique de la zone pellucide des ovules de chatte. *C. R. Soc. Biol., Paris*, **153**, 893–4.

LEWIS, W. & WRIGHT, E. (1935). On the early development of the mouse egg. *Contr. Embryol. Carneg. Instn*, **25**, 113–44.

LOWRY, O. (1953). The quantitative histochemistry of the brain. I: Histological sampling. *J. Histochem. cytochem.* **1**, 420–8.

LOWRY, O., ROSEBROUGH, N., FARR, A. & RANDALL, R. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–75.

MCLAREN, A. & MICHIE, D. (1956). Studies on the transfer of fertilized mouse eggs to uterine foster mothers. I: Factors affecting the implantation and survival of native and transferred eggs. *J. exp. Biol.* **33**, 394–416.

MINTZ, B. (1962). Experimental study of the developing mammalian egg: Removal of the zona pellucida. *Science*, **138**, 594–5.

NOMOTO, M., NARAHASHI, Y. & MURAKAMI, M. (1960a). A proteolytic enzyme of *Streptomyces griseus.* VI. Hydrolysis of protein by *Streptomyces griseus* protease. *J. Biochem, Tokyo*, **48**, 593–602.

NOMOTO, M., NARAHASHI, Y. & MURAKAMI, M. (1960b). A proteolytic enzyme of *Streptomyces griseus*. VII. Substrate specificity of *Streptomyces griseus* protease. *J. Biochem., Tokyo*, **48**, 906–18.

RUNNER, M. (1950). Induced ovulations in immature mice as a source of material for studies on mammalian eggs. *Anat. Rec.* **106**, 313–14.

SMITHBERG, M. (1953). The effect of different proteolytic enzymes on the zona pellucida of mouse ova. *Anat. Rec.* **117**, 554.

SOTELO, J. & PORTER, K. (1959). An electron microscope study of the rat ovum. *J. biophys. biochem. Cytol.* **5**, 327–42.

SPONSLER, O. & BATH, J. (1942). Molecular structure in protoplasm. In *The Structure of Protoplasm* (ed. W. Seifriz). Ames: Iowa State College Press.

STARY, Z. (1959). Mucosaccharides and glycoproteins: chemistry and physiopathology. *Ergebn. Physiol.* **50**, 174–408.

WEISS, L. (1958). The effects of trypsin on the size, viability and dry mass of sarcoma 37 cells. *Exp. Cell Res.* **14**, 80–3.

WISLOCKI, G., BUNTING, H. & DEMPSEY, E. (1947). Metachromasia in mammalian tissues and its relationship to mucopolysaccharides. *Amer. J. Anat.* **81**, 1–38.

(*Manuscript received* 21*st September* 1963)