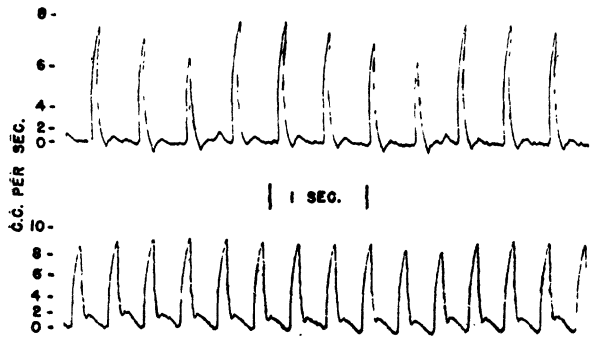

FIG. 5. Records of flow in the abdominal aorta in two dogs after recovery from anesthesia. The upper record is from an older animal which on autopsy was found to have arteriolar disease. This animal delivers his entire stroke volume in about one-fifth the time required for a cardiac cycle.

former flowmeters have greater stability and are not affected by cooling at low flows. Since they are incorporated in a straight tube, they may be easier to insert in certain vessels.

Differential transformer flowmeters require a stable source of alternating current for the primary. The output of the secondary is amplified and rectified by conventional techniques. Where only two windings are employed, these may be connected as adjacent legs of an inductance bridge.

The flowmeters illustrated have been constructed for the larger arterial vessels of the dog, but components may be varied in size for other vessels. They have been successfully employed in the abdominal aorta of the dog under anesthesia and in two animals have been placed in the abdominal aorta and have functioned for three and five days, respectively, after operation (Fig. 5).

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Ribonucleic Acid and Protein in the Growing Oocytes of *Triturus pyrrhogaster*

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The growing oocytes of Amphibia present a very convenient material for the study of cell growth, and their yolk formation has been frequently studied in connection with ribonucleic acid (*1–5*). However, before we can discuss the chemical mechanism of yolk formation it seems necessary to accumulate exact data by different approaches. In the present paper the first sets of our experiments along this line will be reported, in which quantitative estimation of RNA and protein was performed on growing oocytes of *Triturus pyrrhogaster*, and the data obtained were compared with the histochemical picture of the same subject. Throughout the experiments rapidly growing winter oocytes before maturation were used.

Histochemical. As the fixative absolute alcohol, Zenker's fluid or 10% formalin was used. Sections were stained with methyl green-pyronin or toluidin blue. In the control series sections treated with ribonuclease or hot trichloroacetic acid were stained. The alcohol-fixed material gave the most beautiful staining with these dyes.

In young oocytes smaller than 0.05 mm^3, the cytoplasm was homogeneously and deeply stained, whereas the nuclear sap showed a granular staining. Many small nucleoli,[2] showing intensive basophilia, were attached to the internal surface of the nuclear membrane. In oocytes of about 0.7 mm^3 the germinal vesicle showed hardly any staining except in the nucleoli. The strongly basophilic nucleoli, which were somewhat larger in size and contained one or more refractory vacuoles, were found adjacent to the nuclear membrane. A relatively intense staining of the cytoplasm was observed around the germinal vesicle. In oocytes of about 2 mm^3 a very faint cytoplasmic staining was found on the external surface of the germinal vesicle. Most of the nucleoli appeared less basophilic, showing the above mentioned vacuoles and an irregular contour. In oocytes of 3 mm^3 the cytoplasm was fully laden with yolk granules, and they had almost completely lost their basophilia. The faintly stained nucleoli were found in the center of the germinal vesicle, whereas none were found in the periphery. This condition of both cytoplasm and germinal vesicle was observed also in later stages of the growth period. With respect to the behavior of the nucleoli, all fixatives gave approximately the same results. Our results are in good accord with the observations of Duryee (*7*) on the living oocytes of the same species.

The following facts made it probable that the basophilia of above-mentioned structures was due to RNA: (a) the stainability with pyronin and toluidin blue was completely lost by treating sections with crystalline ribonuclease prepared after the method of McDonald (*8*) or by extracting them with 5% trichloroacetic acid at 90° C for 15 min; (b) they were always Feulgen-negative. We have been careful not to accept such histochemical evidence as absolutely conclusive.

The results obtained on the prepared sections were essentially in agreement with those of Brachet (*1–4*) and Wittek (*5*). As repeatedly emphasized by Brachet,

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[2] Some doubt has been expressed about identifying these structures as nucleoli (*6*). Without going into a discussion of this point, we provisionally adopt here the usual terminology.

these pictures give us an impression that the cytoplasmic RNA of the growing oocyte was rapidly decreasing during oogenesis. Similar results were also reported for oogenesis of other forms (*9–12*), and have been discussed in relation to the yolk-protein synthesis (*5*). However, the volume of the oocyte at the last growth stage under our examination was about 100 times as large as that of the initial stage. Therefore the decrease in concentration, judged by the stainability, does not necessarily mean a decrease in the absolute amount of RNA of an oocyte during the growth period.

Biochemical. The ovaries were removed from one to three females and immediately fixed with ice-chilled absolute alcohol. The oocytes were torn off from the ovarian epithelium, divided according to their diameter into groups of 15 to 60, and homogenized in ice-chilled absolute alcohol according to the directions of Ogur and Rosen (*13*).[3] Fractionation and photometric

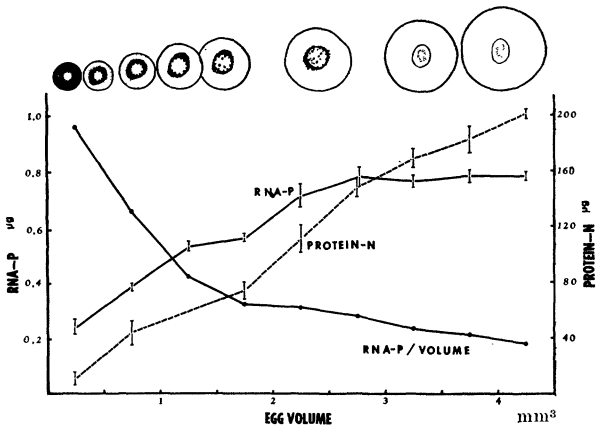

FIG. 1. RNA-P/oocyte, RNA-P/volume, and protein-N/oocyte compared with diagrams of basophilia during the growth of oocyte of *Triturus pyrrhogaster*. Each plotted value of RNA-P is the mean of 7 to 14 separate determinations. The value for protein-N is based on two to four separate determinations. Standard error is indicated for each value of RNA-P/oocyte and protein-N/oocyte curves.

estimation of RNA were made according to Schneider (*14*), and the amount of RNA thus obtained was expressed in terms of μg RNA-P/mm³ and of μg RNA-P/oocyte. Data are shown in Fig. 1, which is accompanied by histochemical pictures of each stage examined. As we have no exact knowledge about the temporal sequence of stages of growth of oocytes, obtained values were plotted against the size of oocytes. As will be seen from Fig. 1 the amount of RNA/cell progressively increased with its growth in the early phase of the period studied. However, beyond a certain point, the value remained almost constant in spite of increase in volume. On the other hand the concentration curve (RNA/mm³) fell sharply during the earlier phase and then declined gradually.

The general trend of the basophilia found in the sections of the growing oocytes seems to reflect the concentration curve of RNA-P. But closer comparison of this curve with the histochemical pictures suggests that it is very dangerous to predict any quantitative relation from the histochemical pictures alone. On the other hand, the diagram shows that the phase of increase in absolute amount of RNA coincides with the stages in which the strongly stainable nucleoli are applied to the internal surface of the nuclear membrane, whereas no increase in the absolute amount of RNA occurs in the later stages where the less stainable nucleoli are found only in the central part of the nucleus.

Brachet (*2*) reported that the young oocyte has a higher RNA content compared with the grown oocyte, calculating its value in terms of concentration per unit weight. On the other hand Villee and Duryee (*15*) found 2.05 times as much RNA-P in the larger oocyte as in the smaller one.[4] Our results also show that despite the apparent decrease of its concentration due to the increase in the volume of the cell, the absolute amount of RNA per oocyte does increase in the earlier phase of the growth period and does not decrease throughout the entire period.

The above estimations were carried out on the oocytes with the follicular epithelium. Although our histochemical study showed no detectable amount of RNA in the follicular epithelium, it seemed necessary to know whether the presence of the latter does not interfere with the estimation of the amount of RNA in the oocyte. To check this point the follicular epithelium was operatively removed in 1 *M* NaCl containing 0.01 *M* citrate, and the naked oocyte thus obtained was compared for RNA content with oocyte with the intact follicular epithelium. No appreciable difference was found between the two series.

Further, the estimation of RNA in the operatively separated nucleus and cytoplasm of the grown oocyte was carried out in a preliminary series and more than 98% of total RNA-P was recovered in the cytoplasmic fraction. This again seems to show that despite its apparent lack of basophilia, the cytoplasm of the grown oocyte does contain an appreciable amount of RNA.

Quantitative measurement of total protein-N was undertaken by the method of Levy and Palmer (*17*) on the "Schneider residue." As shown in Fig. 1, the curve of total protein-N/oocyte rises approximately linearly with the increase of cell volume. If we compare the curve of protein-N with that of RNA-P no simple correlation seems to exist. RNA increase per cell stops before the maximum amount of protein is accumulated. Logarithmic treatment of the data indicates that the rate of increase of RNA per cell is

[3] Chilled alcohol was chosen because of the convenience for tearing the oocytes from the ovarian tissue. The curve obtained from several experiments in which fresh oocytes were fixed and homogenized in cold 10% TCA showed no essential difference from that of alcohol-treated material. The method of differential estimation of RNA and DNA of Ogur and Rosen (*13*) was not suited to our material owing to incomplete separation of the two substances.

[4] In his recent publication, which was received after the completion of this paper, Steinert (*16*) pointed out that RNA in the oocyte of *Rana fusca* increases during its growth.

lower than that of protein accumulation throughout the growth period and that it has a tendency to reduce its value in the later phase of this period.

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Filter Paper Electrophoresis of Avian Serum Proteins[1]

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The serum proteins of the fowl have been studied by the classical Tiselius' method (*1, 2*), but not, so far as we are aware, by the method of filter paper electrophoresis described by Durrum (*3*) and by Cremer and Tiselius (*4*). The present communication describes preliminary results of studies of fowl sera by filter paper electrophoresis, including studies on the highly lipemic sera of laying and estrogenized birds. Kunkel and Slater (*5*) have pointed out that filter paper electrophoresis may have special advantages for studying lipoprotein components of serum.

The technique was essentially that of Durrum (*3*), as modified by Flynn and De Mayo (*6*). Strips of Whatman 3 MM paper $2\frac{1}{2} \times 16$ in. were used. The Veronal buffer (pH 8.6, ionic strength 0.05) was allowed to rise from both ends of the paper by capillarity until a narrow transverse zone near the anodic end remained unwetted. From 0.1 to 0.2 ml of serum was applied by micropipet to the paper along this zone. The advancing fronts of the buffer were then allowed to come together along the line of application of the serum and before the serum could dry on the paper. This avoided undue diffusion of the proteins, a difficulty which may be accentuated when relatively large amounts of serum (e.g., 0.1–0.2 ml) are applied directly to fully wetted paper strips.

The potential was maintained at 100 v, giving a total current of 8.5 ma when there were 5 strips in the chamber. This current would appear to be smaller than that used by most other workers. A low current reduces trouble from heating and from flow of the buffer. Some (*7, 8*) have described movement of the γ globulin fraction of serum in the opposite direction to the other fractions relative to the place of application. This apparently anomalous movement, due to electroendosomis, is probably accentuated by high currents; it has frequently been absent in this laboratory when using low currents. Control of evaporation and temperature by keeping the papers immersed in a bath of nonaqueous solvent (*4*) was considered inadvisable for studies involving lipoproteins, especially those of the sera of laying or estrogenized fowl. Electrophoresis was usually continued for 24 hr or occasionally longer. Under these conditions the fastest moving major component (the albumin) moved 15–16.5 cm from the zone of application. The drying, staining, and quantitative determination of the dyestuff were performed as described by Flynn and De Mayo (*6*) using bromophenol blue as stain.[3] Lipids were located by exposing the papers to the vapor from a solution of 1% osmic acid in 1% chromic acid. Substances positive to the Liebermann-Burchard reaction were located by immersing papers for a few moments in a reagent made of 10 parts chloroform, 10 parts acetic anhydride, and 1 part concentrated sulfuric acid, and then laying the papers on a glass

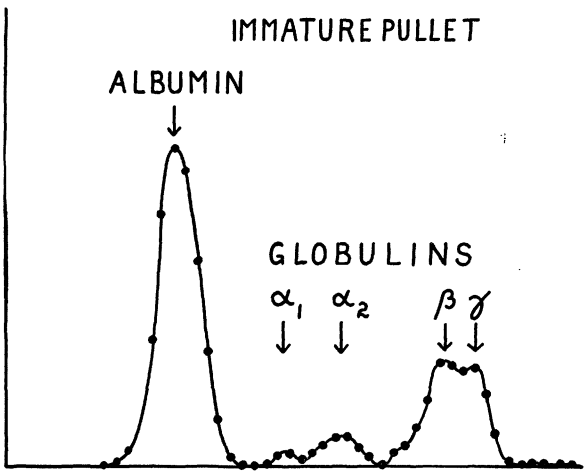

FIG. 1. Quantitative data for paper electrophoresis of avian sera. Vertical axis is optical density. Papers cut in 5-mm transverse strips. A. Immature cockerel, untreated. B. Immature pullet, untreated. C. Turkey hen, nonlaying. D. Normal human.

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[3] *Added in proof.* In our experience, the staining of protein with this dye, and with naphthalene black, was confined to the superficial layers of the paper.