within the nodules. The demarcation of these nodules from the surrounding liver tissue was not sharp. Early changes appeared near the portal triads. They were alterations of the proliferating smallest bile ducts, characterized by heightening of the bile duct epithelium with hyperchromasia, piling up of the cells, and PAS reaction (not given otherwise by proliferating bile ducts) (Fig. 1B). This change occasionally involved only one segment of the wall of the bile duct.

The hepatomas, also usually multiple, consisted of nodules up to 2 mm in diameter which, on the greatest part of their circumference, were sharply demarcated from the surrounding liver tissue (Fig. 1C). The nuclei of the cells were, as a rule, large, vesicular, and showed huge nucleoli. The abundant cytoplasm was loose and eosinophilic. The cells were arranged in several cell thick plates with few capillaries and Kupffer cells between them (Fig. 1D). The nodules contrasted sharply to the liver tissue outside in which the inflammatory and fibrotic changes were marked and the liver cells had a denser cytoplasm and an irregular arrangement. The early stages of these nodules were small groups of liver cells (5 to 8 in a section) which represented focal multiplication of the liver cells so that the plates became several cells thick. The liver cells in these small groups resembled each other but varied from the surrounding tissue.

Hepatomatous and cholangiomatous nodules were found within the same liver. The pancreas of the animals showed severe fibrosis, atrophy of the acini, and vacuolization of the islet cells. These lesions represented a further advance of lesions previously described (*6, 8, 9*).

It appears, thus, that feeding diets containing an amino acid antagonist produces, in relatively short time and rather consistently, small multiple hepatic tumors composed of either liver cells or bile ducts varying in character from those in the surrounding tissue. The precursor stages of the nodule formation can be prevented or corrected by methionine administration. Although abnormal growth and anaplasia are seen histologically, it cannot be determined that the tumors represent malignant growths. No agreement is found in the literature as to whether the lesion called cholangiofibrosis or cholangioma represents neoplasia at all; this is reflected in the variety of names chosen for this lesion which is characteristically produced by carcinogenic dyes. It also cannot be ascertained whether the tumor formation is caused by ethionine directly or results from the excessive regeneration due to persisting injury to liver cells and bile ducts associated with diffuse fibrosis.

References

1. FARBER, E., SIMPSON, M. V., and TARVER, H. *J. Biol. Chem.*, **182**, 91 (1950).
2. STEKOL, J. A., and WEISS, K. *Ibid.*, **179**, 1049 (1949).
3. FARBER, E., KOCH-WESER, D., and POPPER, H. *Endocrinology*, **48**, 205 (1951).
4. KOCH-WESER, D., FARBER, E., and POPPER, H. *Am. Med. Assoc. Arch. Pathol.*, **51**, 498 (1951).
5. FARBER, E. Thesis, University of California (1949).
6. KOCH-WESER, D., and POPPER, H. *Proc. Soc. Exptl. Biol. Med.*, **79**, 34 (1952).
7. POPPER, H., HUERGA, J. DE LA, and KOCH-WESER, D. *Am. J. Pathol.*, **28**, 518 (1952).
8. FARBER, E., and POPPER, H. *Proc. Soc. Exptl. Biol. Med.*, **74**, 838 (1950).
9. GOLDBERG, R. C., and CHAIKOFF, I. L. *Am. Med. Assoc. Arch. Pathol.*, **52**, 230 (1951).

Manuscript received January 12, 1953.

Compact Flowmeters for Use in the Unanesthetized Animal, an Electronic Version of Chauveau's Hemodrometer[1]

Allen M. Scher, Thomas H. Weigert, and Allen C. Young

Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle

Certain characteristics are desirable in a flowmeter for use in animal experimentation. These include (1) high sensitivity, (2) low resistance, (3) a regular and reproducible calibration, preferably linear, and (4) ease of use. Attempts to achieve these have extended over more than a century. Probably the first successful instrument was Chauveau's hemodrometer (*1*). The essential feature was hit upon while watching needles thrust into arteries. His first device consisted of a straight cannula with a rubber membrane as part of the wall. A lever extended from the center of the stream through this membrane, and movements of the lever were read against a calibrated scale. On a later

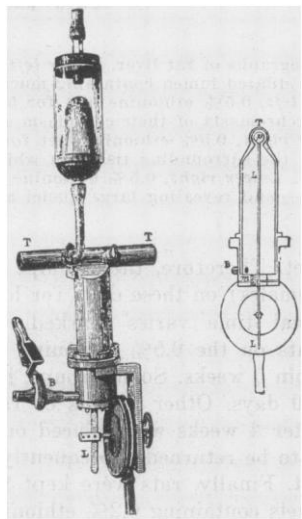

FIG. 1. Chauveau's hemodrometer as diagrammed by Luciani (*5*). The figure on the left shows the entire instrument, the cross section on the right is of the inverted, lower portion of the instrument. S, a sphygmoscope used to record the blood pressure; TT, a tube used to cannulate; L, a lever suspended by a rubber membrane at m. The lower end of the lever within the stream carries a paddlelike obstruction, while the outer end moves a tambour connected by tubing to a recording tambour.

[1] Aided by grants from the State of Washington Research Fund for Biology and Medicine and the National Heart Institute, USPHS.

modification (*2*), the hemodromograph (Fig. 1), the lever was connected to a recording tambour. With this it was first noted that blood was squeezed backward in the coronary arteries by ventricular contraction. The two cannula flowmeters to be described here prove to be in principle electronic versions of Chauveau's instrument of nearly a hundred years ago.

The first type of flowmeter consists of a T-shaped stainless steel tube. The stem or side branch of the T makes a friction fit with the body of an RCA 5734 vacuum tube (mechanoelectronic transducer) and the cross piece is used to cannulate and carries the flow to be measured (Fig. 2). An obstruction to flow lies in this crosspiece and is fastened only to the moving anode of the vacuum tube. Pressures exerted on the obstruction by the stream will cause movement of the sensing element and alter the output of the vacuum tube. A power supply, plate resistor, and meter or oscillograph are the only additional equipment necessary.

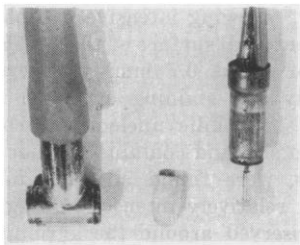

FIG. 2. A flowmeter employing the RCA 5734 tube. On the right the tube with a streamlined obstruction, in the center a flat paddle, and on the left an assembled flowmeter in stainless steel T-cannula. Diameter of the tube at its widest point is 0.375 in.

Two types of obstruction to flow have been used. One consists of a paddle of stainless steel which is placed across the stream to give "form" drag. The second is a streamlined rod or tube of plastic which offers "skin" or viscous drag. The paddle flowmeters may be made extremely sensitive by increasing the paddle cross section, but the calibration is that of a "V^2" device, that is, the deflection of the paddle is proportional to the square of the velocity. This characteristic results in severe distortion of wave forms and requires replotting of records where pulsatile flow is recorded. Viscous drag flowmeters are less sensitive, but their calibration approaches linearity (deflection = $kv^{1.2}$), and they offer less resistance to flow. The 5734 tube has ample sensitivity for use in a viscous drag flowmeter.

The mechanoelectronic transducers have been found to vary somewhat in characteristics. At very low flows they will change output due to cooling. For this reason plastic cannulas were considered less useful. It is possible that in animal use plastic will be more satisfactory than metal. In calibration the whole assembly was immersed in fluid, which minimized cooling effects. The vagaries of the vacuum tube are more evident with less sensitive flowmeters. Typical calibration curves are shown (Fig. 3).

A second group of flowmeters has been constructed

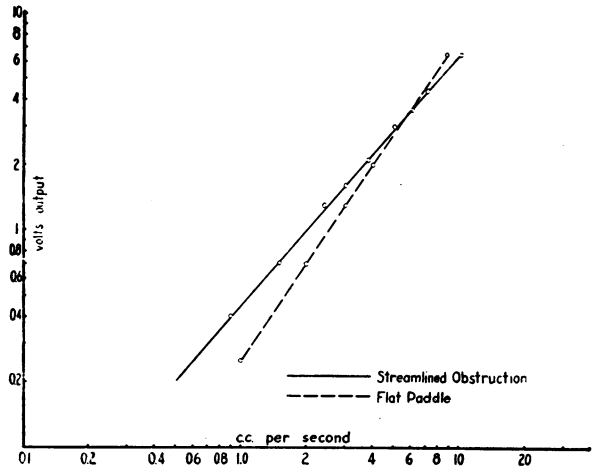

FIG. 3. Calibration curves for two flowmeters employing the 5734 tube with flat and streamlined obstructions.

in which the obstruction was of ferromagnetic material, and a plastic cannula wall incorporated windings whose inductance was altered by the movement of the obstruction. Some of these had both primary and secondary windings so that the whole constituted a differential transformer. With this design, the obstruction may consist of a flexible paddle, or a spring-suspended disk or cylinder. The windings may take several different shapes and the illustration presented (Fig. 4) is schematic for many that may be constructed. In these flowmeters, sensitivity is determined by the number of turns of wire, the magnetic permeability and movement of the element and its distance from the windings. A ferromagnetic frame for the windings will increase the sensitivity by increasing the amount of flux passing through the obstruction. Despite the use of all possible measures to achieve sensitivity, these flowmeters have never been sensitive enough to employ a streamlined obstruction with flow velocities such as are found in the dog's abdominal aorta. Although characterized by lower sensitivity than flowmeters using the 5734 tube, differential trans-

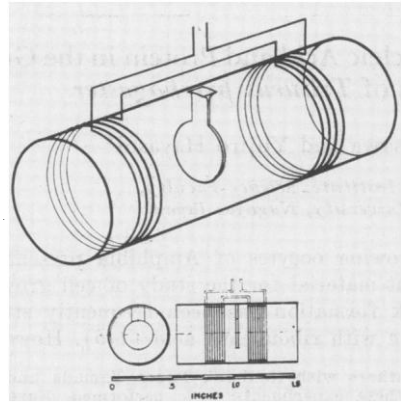

FIG. 4. Schematic diagram of a flowmeter in which a moving ferromagnetic obstruction alters the inductance of windings around a tube.

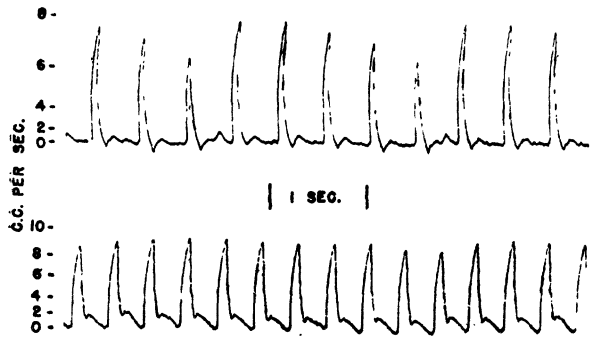

FIG. 5. Records of flow in the abdominal aorta in two dogs after recovery from anesthesia. The upper record is from an older animal which on autopsy was found to have arteriolar disease. This animal delivers his entire stroke volume in about one-fifth the time required for a cardiac cycle.

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).

References

1. CHAUVEAU, A., BERTOLUS, G., and LAROYENNE, L. *Journal de la Physiologie de l'homme et des animaux*, **3**, 695 (1860).
2. LORTET, L. *Recherches sur la vitesse du cours de sang dans les artères du cheval au moyen d'un nouvel hémadromographe*, (Monograph). Paris: J. B. Ballière (1867).
3. LUCIANI, L. *Human Physiology*, Vol. 1 (translated by F. A. Welby). London: Macmillan, 274 (1911).

Manuscript received February 9, 1953.

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

Syozo Osawa and Yujiro Hayashi[1]

Biological Institute, Science Faculty, Nagoya University, Nagoya, Japan

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,

[1] The authors wish to thank Tuneo Yamada under whose guidance these experiments were performed. Further, they express their appreciation to Barry Commoner, Eugene Roberts, and Florence Moog for critical reading of the manuscript. The investigation was aided by the Science Research Expenditure of the Department of Education.

[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.