COMMENTS
by Readers

The article by E. Margoliash (*Science*, April 4, pp. 369–370) suggests the following comment:

Difficulties of obtaining sterile preparations from nonsterile treated raw tissue extracts have been the main hindrance to the study of the effect of these substances on wound healing in tissue cultures and in medicine. In his paper Margoliash recommends a "new method" of sterilization of the dry proteins. This method, involving the use of acetone or benzene, was originally designed long ago. It was described by both the undersigned and T. Astrup (*Acta Physiol. Scand.*, 1941, **3**, 54; *Pflüg. Arch. ges. Physiol.*, 1943, **247**, 34) and later adopted by J. Davidson and C. Waymouth (*Biochem. J.*, 1945, **39**, 188) in their work on nucleoproteins and cell multiplication.

Margoliash has failed to give due reference to the author of the method, in spite of the fact that the particular articles cited were sent to him long ago. (ALBERT FISCHER, *Biological Institute, Carlsberg Foundation, Copenhagen, Denmark.*)

The results of creatinine, hippurate, and inulin clearance estimations in rats have been reported recently by M. Friedman (*Amer. J. Physiol.*, 1947, **148**, 387), who concluded that these clearances "vary directly with the urine flow." This conclusion differs from that of Dicker and Heller (*J. Physiol.*, 1945, **103**, 449) in that no such correlation was observed in their experiments comprising 134 estimations of inulin clearances in rats. It would therefore seem of interest to comment on Friedman's technique and his results.

At variance with the technique of Dicker and Heller, Friedman anaesthetized his rats immediately before the beginning of the urine-collecting period. The anaesthetic is not specified, but he states that "the clearances were not influenced by the very brief duration of anaesthesia." No experimental evidence for this claim is given. However, judging from the disturbance of the water metabolism in rats anaesthetized with chloroform or ether (H. Heller and F. H. Smirk. *J. Physiol.*, 1932, **76**, 292), and considering the results of clearance experiments on anaesthetized dogs (A. C. Corcoran and I. H. Page. *Amer. J. Physiol.*, 1943, **140**, 234; F. N. Craig, F. E. Visscher, and C. R. Houck. *Amer. J. Physiol.*, 1945, **143**, 108), it would seem hazardous to dismiss so lightly the possible effects of anaesthesia. But, even assuming that light anaesthesia did not interfere with the results of Friedman's clearance estimations on rats, it should be pointed out that his animals were disturbed further (a) by withdrawal of up to 1.75 cc. of blood from the tail immediately before the beginning of the urine-collecting period, (b) by a tourniquet placed on the stump of the tail and kept in position in the unanaesthetized animal, and (c) by handling (administration of water and injections) during the urine-collecting period. The adverse effects of these procedures on kidney function can be demonstrated from the author's own figures: Normal rats (given 5 cc. water/100 grams body weight) excrete about 3 cc./100 grams in two hours. In the case of Friedman's animals, 26 out of 101 excreted less than 1 cc./100 grams in two hours, and 31 less than 2 cc. in two hours, indicating that many of his rats suffered from a marked inhibition of diuresis. It was probably because of this poor urinary output in a majority of his animals that Friedman decided to extend his urine-collecting periods to two hours. A period of this length involves unnecessarily large changes of the plasma concentration of the test substance. Much shorter periods are therefore commonly used. Estimations of the plasma concentration of the test substance at the beginning and end of a two-hour period are unlikely to compensate for this disadvantage, especially when, as in Friedman's case, creatinine was given a second and third time *after* the initial blood sample was taken.

Taking the results of Friedman's experiments as a whole, it would seem that at very low ranges of urine flow, *i.e.* in animals suffering from inhibition of diuresis due probably to pain, anaesthesia, etc., variations of clearance with urine flow may occur. [The effects of increased secretion of the posterior pituitary hormones (S. E. Dicker and H. Heller. *J. Physiol.*, 1946, **104**, 353) and of adrenaline should be considered in this connection.] However, at higher rates of urine flow (1–2 cc./100 grams/hour) not only are Friedman's figures for inulin clearances similar to ours but it can also be shown that they bear no clear relation to the rate of diuresis. A statistical comparison between his figures at urine flows of 1–1.5 cc./100 grams/hour and those at 1.5–2.0 cc./100 grams/hour shows no significant difference (Fisher's $t = 1.399$, $P > 0.1$). It seems probable, therefore, that the relation between inulin clearance (= glomerular filtration rate) and rate of urine flow in the rat is similar to that in the dog, in which very low rates of flow are also accompanied by low inulin clearances but the rate of glomerular filtration is regarded as "essentially constant and unrelated to the rate of urine flow in the ordinary experimental range of the latter" (J. A. Shannon. *Amer. J. Physiol.*, 1936, **117**, 206; H. W. Smith. *The physiology of the kidney*. London: Oxford Univ. Press. P. 64). (S. E. DICKER and H. HELLER, *Department of Pharmacology, University of Bristol.*)

In presenting the case for a National Institute of Biology (*Science*, May 30, p. 559) Robert F. Griggs tabulates and classifies in great detail the array of divers organizations that make up the biology fraternity—from the Society of Agronomy to the Society of Zoologists. Yet nowhere in Dr. Griggs's paper is as much as a passing mention made of the teachers of biology in our secondary schools, a group organized locally, regionally, and nationally. These men and women are devoting their lives to the dissemination of biological knowledge and to the development of an appreciation of the role that biological knowledge can play in the lives of individuals and in society.

Among his concluding remarks Dr. Griggs states: "All biology must organize to lead the public in its thinking on biological matters." One wonders how far the public is going to be led if we leave out of consideration entirely our first line of contact with the public at its most formative stage. (ZACHARIAH SUBARSKY, *Department of Biology, The Bronx High School of Science.*)