COMMENTS
by Readers

In a recent communication to this column (*Science*, August 5, p. 127) Drs. Dicker and Heller critically reviewed a recent paper of the writer dealing with the creatinine, inulin, and hippurate clearance of the rat (*Amer. J. Physiol.*, 1947, **148**, 387). It was their belief that (1) the preliminary and very brief (less than 5 minutes) ether anesthesia given to the rats preceding our clearance studies, (2) handling of rats during the clearance, (3) withdrawal of 1.0–1.75 cc. of blood from the rats before the clearance, and (4) the possible variation of the blood creatinine during a possible two-hour collection period, all tended to give us results which might not be comparable to those obtained by Drs. Dicker and Heller, who employed collection methods which they believed to be more physiological.

If factors (1), (2), and (3) above were operating in our clearance studies, they acted as the authors themselves have stated (*J. Physiol.*, 1945, **103**, 449), namely, to depress renal clearances. This, then, makes it difficult to understand why our average inulin clearance (19.1 cc.) at our lowest rate of urine flow was approximately the same as the average inulin clearance (21.06 cc.) of all of their clearances and why our average inulin clearance (41.1 cc.) at a high rate of urine flow was 90 per cent higher than theirs. The same discrepancy holds for their and our rates of renal plasma flow. In other words, if our technique supposedly depressed clearances, thus making them different than theirs, why are our clearances so much higher?

Concerning the fourth factor (the creatinine variation), we found, in preliminary determinations taken every 15 minutes during the collection period, that the blood creatinine remained relatively unchanged. Drs. Dicker and Heller assume that we conducted a two-hour collection because of small urine collections. We stated in our paper that the two-hour collection was designed to avoid the necessity of making clearance calculations on total collection volumes which might not exceed 0.07 cc. of urine, as occurred in some of Dicker and Heller's experiments. We further stated, and still believe, that their clearances were erroneously low because of possible urine losses attending such small collections.

Drs. Dicker and Heller are referred to the clearance results of the rat as given by Drs. Braun-Menendez and Chiodi (*Rev. Soc. Arg. Biol.*, 1946, **23**, 314). In this independent study of inulin and diodrast clearances of the rat, clearances were obtained by a technique differing from ours. Nevertheless, Dr. Braun-Menendez has informed me (as a review of his article will also demonstrate) that his clearances were almost identical with ours. Furthermore, both he and Dr. Chiodi were convinced that their clearance values varied with the urine flow. (MEYER FRIEDMAN, *Mount Zion Hospital, San Francisco, California.*)

In the years to come American scientists will presumably wish to participate actively in international conferences, some of which may be called on fairly short notice. The story that follows may serve to illustrate the kinds of obstacles that one may encounter because of passport difficulties. We must find ways of eliminating obstacles of this sort.

Early in August I received an official confirmation of a provisional invitation for me to attend a three-day conference called for September 11–13 by the Committee on Science and Its Social Relations (CSSR). This confirmation was in the form of a telegram from Dr. F. J. M. Stratton, chairman of the CSSR and also secretary of the International Council of Scientific Unions (of which the CSSR is a committee).

Certain preliminaries had to be taken care of, and, on August 18, I presented in person at the office of the Passport Division of the State Department in Washington, D. C., a fully-documented passport application. A passport that had originally been issued to me in 1938, and which was recalled as a matter of routine in 1941, was on file in Washington. The clerk who received my application assured me that my passport would be issued within a week. I informed him that I was making air reservations to leave September 6 or 7.

Accompanying the passport application was an official personal letter of endorsement from Dr. Detlev W. Bronk, chairman of the National Research Council, in which he requested the issuance of a special passport. In this letter Dr. Bronk pointed out that as a part of this trip to Europe I would also stop in Paris at UNESCO Headquarters, a visit that was highly desirable for me as chairman of the NRC Committee on UNESCO.

I was preparing to leave on September 7, but when, after two weeks, no passport came, I went (again in person) to the Passport Division and inquired about the reasons for the delay. The reception given to me was kind and courteous, but I was informed by the assistant chief of the Passport Division that he could not issue a passport as long as "official clearance" had not been obtained. He promised to send me the passport himself, the moment the needed clearance would come through.

The NRC then renewed its efforts to obtain a passport in time for me to proceed to the London meeting. I informed Dr. Harlow Shapley of the unexpected delays, and he sent a telegram to the Secretary of State, urging that a passport be issued to me right away. Dr. Shapley sent this telegram in his capacity of president of the AAAS.

A personal inquiry by me to the head of the Federal Bureau of Investigation revealed that the issuing of the required clearance was wholly in the hands of the State Department.

On September 9 I talked by telephone with the assistant chief of the Passport Division, who held out hopes that a passport might come through later that day or early the next day. He offered to do everything possible to speed me on my way if the needed clearance were to come through on September 9 or 10. The "security investigation" was, however, not completed in time, and no passport was issued. On September 10 I had to telegraph to London that I would be unable to attend the London meeting.

I understand that another scientist

who was invited to attend the London meeting of the CSSR was also prevented by passport difficulties from going. The readers can judge for themselves what sort of impression our European colleagues must have formed of the importance which our State Department attaches to international scientific conferences. (BART J. BOK, *associate director, Harvard Observatory.*)

Current interest in the therapeutic use of cytochrome C is widespread as a result of a series of papers by S. Proger and associates (*Science*, October 25, 1946, pp. 389–390; *J. clin. Invest.*, 1945, **24**, 864). In an attempt to provide a rational basis for their therapeutic studies, these workers drew certain conclusions which we feel are unjustified. It is understandable that such erroneous conclusions could be drawn, but it is undesirable to have them go unchallenged. The points at issue are as follows:

(1) Proger, *et al.* stated (*Science*, October 25) that "the organs normally contain considerably more cytochrome oxidase than can be activated by the cytochrome C present," based upon our data for cytochrome C content of organs (V. R. Potter and K. P. DuBois. *J. biol. Chem.*, 1943, **142**, 416) and cytochrome C requirement for *in vitro* assay of cytochrome oxidase (W. C. Schneider and V. R. Potter. *J. biol. Chem.*, 1943, **149**, 217). This conclusion is not permissible because the amount of cytochrome required in the assay system is not an indication of how much is needed in the cell and was not intended to be. Proger, *et al.* apparently overlooked the fact that the substrate for cytochrome oxidase is *reduced* cytochrome C, and that the amount of *reduced* cytochrome available to cytochrome oxidase is a function not only of the total cytochrome C present but of the *rate of reduction*. In the assay system this reduction is nonenzymatic and slow; hence, large amounts of cytochrome are used. In the cell the reduction is enzymatic. *Thus, there is no evidence to indicate that cytochrome oxidase needs more cytochrome C than it has available in the cell.*

(2) Proger, *et al.* stated in both articles cited that the cytochrome content of blood and organs was increased following cytochrome C injection. The method used was that of Potter and DuBois. This method does not permit one to decide whether the cytochrome C has penetrated to the inside of the cells or whether it is in the blood and tissue spaces, and was not claimed to do so. In uninjected animals the blood does not contain cytochrome in significant amounts, but this is obviously not the case in the injected animals. *Thus, there is no evidence that injected cytochrome C reaches the interior of the cells.*

(3) Proger, *et al.* also stated that the addition of cytochrome C to homogenized tissue caused increases in oxygen uptake, and concluded that similar amounts of cytochrome C would produce comparable increases *in vivo*. But we have repeatedly emphasized the fact that when a tissue is homogenized, the cytochrome is "diluted" to an extent that depends upon a variety of factors; the extent of the dilution determines the extent of the "stimulation" when cytochrome C is added back. In the intact cells, the cytochrome C is apparently localized in the particles that contain cytochrome oxidase (W. C. Schneider, A. Claude, and G. H. Hogeboom, to be published). There has been no demonstration that the stimulation of oxygen uptake by cytochrome additions observed in homogenates can be duplicated *in vivo*, although the possibility remains that the factors which are concerned in the dilution of cytochrome in homogenates may occasionally operate *in vivo*.

(4) Proger, *et al.* (*J. biol. Chem.*, 1945, **160**, 233) reported that cytochrome C administration prevented the anoxic depletion of the high-energy phosphate reservoirs of the tissues. To me, this experiment would be decisive if it could be confirmed. Unfortunately, the original experiment was done without the precautions that are necessary to preserve the phosphate compounds (G. A. LePage. *Amer. J. Physiol.*, 1946, **146**, 267), and Scheinberg and Michel (*Science*, April 4, pp. 365–366) have failed to confirm the observation.

(5) There remains the final test, clinical benefit, which we are in no position to judge. We have been advised of two unpublished studies with experimental animals that gave negative results. It is desirable that the findings of Proger, *et al.* be tested by some disinterested group as soon as possible in order to prevent a great deal of unnecessary duplication of effort. At present nearly every major pharmaceutical house is undertaking to prepare cytochrome C. It is not the function of these companies to referee conflicting reports, and if the demand for cytochrome C continues, it will be met. But the demand is not a proof of efficacy.

(6) Finally, it must be noted that sound clinical results will stand regardless of their theoretical basis. It may be that cytochrome C will prove beneficial for reasons as yet unknown. (VAN R. POTTER, *University of Wisconsin Medical School.*)

[The above comment was sent by the author to Dr. Proger for criticism before being submitted for publication. Dr. Proger's reply will appear in next week's issue.]

The unfertilized egg of an oyster is pear shaped. In the ripe, unspawned ovary the eggs are tightly packed and compressed. The diameter of the rounded portion of an egg in the oysters kept at Woods Hole is about 40 μ. Assuming that the egg is a sphere, its volume is equal to $\frac{4}{3}\pi R^3$, or $1.33 \times 3.1416 \times 8{,}000$ μ. The volume of 100,000,000 eggs is therefore only 3.3 cc. A certain correction, probably not exceeding 20 per cent, should be added to this figure to account for the void spaces between the eggs. Since the volume of the body of an adult female oyster (without the shell) varies from 15 to 25 cc., the estimated volume of eggs discharged in one spawning is not unreasonable, for it is known that the oysters lose a considerable portion of their body weight after the discharge of sex products.

In the past, too much significance was given to the number of eggs produced by females. Studies conducted by the U. S. Fish and Wildlife Service show that in Southern waters the spawning season extends from early May to October. It is therefore quite possible that the growth of the ovocytes is a more or less continuous process. This point requires further studies which are being conducted at present by the U. S. Fish and Wildlife Service. Potential fecundity of the oyster has, however, little bearing on the success or failure of reproduction, the latter primarily depending on the survival of the oyster larvae rather than on the initial abundance of spawned eggs.

I believe this brief note answers Mr. Burkenroad's criticism of my paper (*Science*, September 26, p. 290). PAUL S. GALTSOFF, *U. S. Fisheries Station, Woods Hole, Massachusetts.*)