the blood became solidified. The tube was then transferred to a dry-ice box for storage at approximately −50° to −70° C. Fifteen days later the frozen blood was thawed by immersion in a water bath at a temperature of 40°–42° C., and 4 cc. was injected intravenously into patient L. H. Ten days later this recipient of the preserved parasites developed a (rectal) temperature of 40° C., and blood smears showed ring forms of *P. vivax*.

Patient D. M., who had received 10 cc. of infected blood directly from H. K., the original donor, developed a paroxysm 40 hours after inoculation, and many ring forms of *P. vivax* were found in the peripheral blood. After several paroxysms, when the parasite count was approximately 5,000/mm.^{3}, blood from D. M. was withdrawn into citrate. Approximately 2–3 cc. of this citrated blood was transferred to each of several small pyrex glass test tubes, which were closed with cork stoppers; and, as before, the blood was frozen by immersion in alcohol-dry ice mixture, then transferred immediately to a dry-ice box for storage. After a 10-day period of storage this blood was thawed, again by immersion in a water bath at a temperature of 40°–42° C. Several tubes which cracked during either freezing or thawing had to be discarded. About 4 cc. of the remaining thawed blood was injected intravenously into patient V. V., who on the 9th and 10th days after inoculation developed a low-grade fever with peaks of 37.8° and 38.0° C., respectively, but parasites were not demonstrated until the 11th day, when the patient had a shaking chill with fever of 41° C.

After two more paroxysms, the parasite density having risen to approximately 1,500/mm.^{3}, V. V. was used as donor for the third experiment. The bleeding, anticoagulant, and freezing process were carried out in the manner previously described, with the exception that small, plastic, screw-capped vials were employed as containers. Freezing and transfer to the storage compartment were completed within ½ hour after bleeding. Fifteen days later the preserved blood was rapidly thawed (as before) and 6 cc., the contents of two vials, inoculated intravenously into each of two neurosyphilitics receiving penicillin therapy. After an interval of 9 days one recipient (E. M.) had onset of periodic episodes of chilliness, headache, and malaise accompanied by slight rises in temperature (maximum, 38.1° C.). The occurrence of this symptom complex followed a tertian pattern, but plasmodia were not demonstrated until the 19th postinoculation day, thick films having been negative on the 10th, 15th, and 17th days. On the 20th day a paroxysm occurred with elevation to 39.4° C.

Removal of the second recipient from the hospital during the incubation period terminated observation. The result of this inoculation is therefore not known.

It should be noted that the freezing and thawing process resulted in almost complete hemolysis of the red blood cells and that the staining characteristics and morphology of the parasites were considerably altered. Of three patients inoculated successfully, one recipient of the hemolyzed blood had a mild chill immediately after injection, with fever to 38.0° C., one had no apparent reaction, and one suffered a simultaneous febrile Herxheimer reaction (penicillin) which masked any unfavorable effect of the inoculum.

Many factors which may influence the success or failure of preservation of human malaria parasites by freezing remain to be studied. Among these are the age of parasites when frozen, the speed of freezing and of thawing, the temperature range during storage, and the anticoagulant used. Studies are continuing to determine the effects of altering these variables, with the objective of developing a practical, reliable method of preserving human plasmodia for long periods.

It is suggested that this method may be used to provide a blood bank of malaria parasites for use in malaria therapy of neurosyphilis, and for preserving strains of human plasmodia for experimental purposes.

Addendum. Since this report was submitted for publication, three additional patients have been inoculated successfully with blood containing *P. vivax* preserved by freezing. In one instance, the blood had been frozen for 37 days.

References

1. Coggeshall, L. T. *Proc. Soc. exp. Biol. Med.*, 1939, **42**, 499–501.
2. Manwell, R. D. *Amer. J. trop. Med.*, 1942, **23**, 123.
3. Manwell, R. D., and Edgett, R. *Amer. J. trop. Med.*, 1943, **23**, 551.
4. Russell, P. F., West, L. S., and Manwell, R. D. *Practical malariology.* New York: W. B. Saunders, 1946. P. 99.
5. Turner, T. B. *J. exp. Med.*, 1938, **67**, 61–78.
6. Weinman, D., and McAllister, J. *Amer. J. Hyg.*, 1947, **45**, 102.
7. Wolfson, F. *Amer. J. Hyg.*, 1945, **42**, 155.

Intraneural Bipolar Stimulation: A New Aid in the Assessment of Nerve Injuries

Frank E. Nulsen and F. H. Lewey

Cushing General Hospital, Framingham, Massachusetts

Clinical observation may sometimes lead to erroneous conclusions concerning regeneration in peripheral nerve injuries. Such instances occur when a patient, despite neurotization of a muscle, remains incapable of voluntary movement or, conversely, when overlap innervation by a neighboring nerve seems to give evidence of regeneration although the nerve in question is actually interrupted.

The extent of regeneration of a nerve may often be clearly ascertained by surgical exploration, with electrical stimulation of the exposed nerve and observation of the reaction in its muscles. A means of obtaining such evidence without an operation has been devised.

Normal peripheral nerves may be excited by electrical stimulation through the intact skin. However, to excite the much less irritable regenerating nerves, stimulation over the skin (or with one electrode within the nerve) must be of such intensity as to result in mass contractions of adjacent muscles. This spread can be avoided and a small stimulus made effective if the current density about the nerve is increased by inserting both electrodes into or near the nerve.

Two sterile #25 hypodermic needles are introduced through the prepared skin, separated by a distance of 1–2 cm., into or close to the nerve in its longitudinal axis. A site of stimulation can be so chosen as to be proximal to the muscles in question and still at an area where the nerve is well defined in its position by surface topography. An effective stimulus is afforded by applying to the two needles a current of less than 5 milliamperes. Alternating and direct currents are equally satisfactory. Any of the usual stimulators may be used; in fact, even the make-brake stimulus delivered by a 1½-volt dry cell in an adjusted flashlight is sufficient. The current is gradually increased until a

maximal contraction is obtained in the innervated muscles. The strength of contraction is estimated or measured. In the absence of motor response the presence of some regenerating nerve fibers at the point of stimulation is indicated by a tapping or burning sensation projected into the skin distribution of the stimulated nerve. If no sensory effect can be elicited, one may assume complete absence of regeneration only after having varied the position of the needles several times to preclude the possibility of poor contact with the nerve.

Intraneural bipolar stimulation has been carried out on over 1,000 patients at Cushing General Hospital during 1945 and 1946. Positive responses were followed by the return of voluntary contraction of variable degree. The absence of response could be confirmed, in most instances, by stimulation of the exposed nerve at the subsequent operation performed because of this finding. The first appearance of muscle contraction on intraneural stimulation permitted calculation of a mean nerve-growth rate of 1.4 mm./day. Long-term follow-up studies have shown that when, in a given patient, the schedule for beginning contraction of various muscles, predicated upon this growth rate, was not maintained within narrow limits, appreciable recovery of function could not be expected by further waiting.

Intraneural stimulation is an objective and easy means of discovering the extent of spontaneous or postsutural regeneration in an injured nerve. Needless surgery is avoided, and necessary surgery is anticipated because of the possibility of determining with greater accuracy and at an earlier time the presence or absence of adequate nerve regeneration.

Interlaboratory Comparison of Measurements of I^{131}

L. F. Curtiss

National Bureau of Standards, Washington, D. C.

Radioactive iodine, I^{131} (8.0 days), is coming into use in increasing quantities in medical research and clinical treatments. It is desirable to have quantitative measurements of this material which are reasonably accurate for many of the purposes for which this isotope is used. Since quantitative procedures for measurements of this type are still in a developmental stage, many of the users as well as the producers have requested some form of intercomparison to determine how accurately measurements are made at present, with the aim of discovering and correcting any serious disagreements.

As a first step in this program the National Bureau of Standards procured a sample of I^{131} from the Clinton Laboratories and prepared from it a number of identical 25-ml. samples which were distributed to approximately 40 hospitals, universities, and similar institutions using this isotope or interested in its measurement. They were requested to measure this sample, which was approximately 1 rutherford (10^6 disintegrations/second) total activity, and to report the results. Replies were received from 30 of these institutions, 26 of which reported results in terms of disintegrations per second. The remaining 4 gave values in counts per second, which could not be interpreted in terms of disintegration rates.

The reported values, plotted in Fig. 1, give some idea of the spread. One value was very high (nearly 100 times the average of the others), which seems to indicate an error in reporting rather than measurement. Neglecting these values, the average value reported was 3.0×10^4 disintegrations/second/ml. of 0.75 rutherford for the 25-ml. sample. The lowest value deviated from the average by 40 per cent and the highest by 80 per cent.

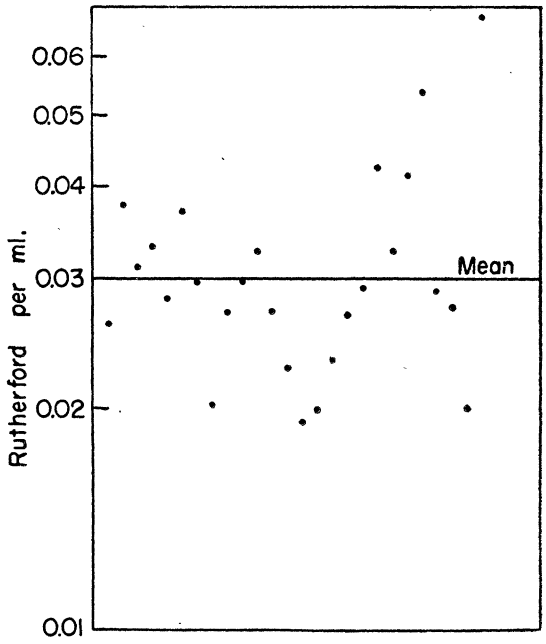

Fig. 1

This preliminary distribution serves only to focus attention on the magnitude of the discrepancies and the need for more accurate standardization. It is certain that much of the variation reported above, in those cases where beta-ray counters were used for measurement, can be reduced by the use of the RaD + E beta-ray standards now available from the National Bureau of Standards. These standards are undoubtedly correct in absolute value to ±5 per cent. By the use of the nominal value, as stated in the certificate accompanying these standards, all counter measurements can be placed on a common basis of comparison to a somewhat higher accuracy.

One sample sent out by us was reported contaminated by a growth of streptococcus on receipt by the cooperating institution. The difficulty of bacterial growth in preparation of P^{32} and I^{131} has been encountered by others. This indicates that all containers should be carefully sterilized before filling, a precaution which we will adopt in the future. Bacterial contamination interferes seriously in measurements which require preparation of aliquots of the original sample.

Since another program of intercomparison is to be started in the immediate future, we would like to hear from other institutions or investigators who may not have been included in the preliminary distribution and who wish to cooperate in the measurement. The method of distribution of calibrated samples is about the most satisfactory way of standardizing in various laboratories measurements for radioisotopes of half-value period too short for the preparation of permanent standards. The National Bureau of Standards proposes to extend this service to other short-period isotopes as the demand arises.