tificate if presented at least thirty minutes before the scheduled time of departure of the train for which it is to be used. Each person presenting an endorsed and validated certificate may purchase a continuous-passage, one-way, return ticket for one half of the regular fare, by the same route as that followed on the trip to New Orleans. Certificates may be validated from December 28, 1931, to January 1, 1932. The last date on which return tickets may be purchased is January 5.

(2) Short limit round-trip winter excursion rates are available from many points in the United States to destinations in Alabama, Florida, Louisiana and Mississippi, and also to Havana, Cuba. These rates have been granted for only certain days in November and December, 1931, and March and April, 1932. During December, 1931, round-trip excursion tickets to New Orleans may be purchased on the following days: 4, 5, 6, 11, 12, 13, 18, 19, 20, 25, 26 and 27. Tickets are limited to 16 days, including date of purchase and actual time required to return to starting-point. The following is a comparison of the cost under the two plans of a round-trip ticket (not including Pullman berth) from Washington, D. C., to New Orleans:

Standard Certificate form (one and one-half fares)	$60.30
Short limit round-trip winter tourist rate	54.30

It is advisable to get information from your local ticket agent about the excursion fares, since there are some points where they are not available. The Canadian eastern lines and the Transcontinental lines are among those that are not authorizing these fares.

CHARLES F. ROOS,
Permanent Secretary

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD OF SUPPLYING STUDENTS WITH NATURAL ENDAMOEBA HISTOLYTICA FROM CULTURES[1]

INSTRUCTORS in protozoology are not always fortunate enough to have an active case of amoebic dysentery available for use in the classroom. Cultures of *Endamoeba histolytica* are commonly used, but the amoebae in culture are much smaller[2] than those encountered in the amoebic stool. It would seem desirable to be able at any time to supply large amoebae and these should also contain red blood cells.

In the course of some experimental studies, irrelevant to the present subject, it was found that the addition of gum arabic to produce a 0.1 per cent. solution in the liquid portion of the ordinary Boeck and Drbohlav medium[3] resulted in a marked increase in the size and number of the amoebae usually present. Further investigation disclosed that the increase in size was probably related to the bacteriostatic action of the gum arabic. As a matter of fact some of the bacteria commonly found in association with the amoebae in culture were not only inhibited but were killed by the use of gum arabic in stronger percentages. The increase in size of the amoebae led one competent observer to remark; "That is the nearest thing to *histolytica* in the stool I have ever noted in cultures."[4] The amoebae, although increased in size, are somewhat slowed in action by the increased viscosity of the fluid.

Usually the addition of red blood cells to cultures of *Endamoeba histolytica* results in the hemolysis of the cells very quickly, with the result that if the cells are ingested only shadows of the cells can be seen within the amoeba. Furthermore, the amoebae, in my experience, seldom ingest the red blood cells under these conditions. In the course of certain immunological studies involving the amoebae, it was found that successful efforts to cause them to ingest red blood cells resulted from the use of the following technique:

Mix the medium well by rotating the culture tubes between the palms of the hands. Combine the liquid portion of ten cultures so treated, place in 10 cc centrifuge tubes and centrifuge at 1,000 r.p.m. for ten minutes. Discard the supernatant liquid, combine the sediment, and add two to five cc of rabbit serum. Mix well with a pipette and add 0.1 cc of guinea-pig or rabbit red blood cells. The latter are obtained by defibrination and centrifugalization. Again centrifuge at 1,000 r.p.m. for ten minutes and place in the water bath for two hours. At the end of this period many of the amoebae will be found to have ingested the cells; some will contain a large number; twenty or more cells may be found within them. The preparations made from sediment were found to be satisfactory up to at least six hours; after this time the cells begin to hemolyse and the amoebae to disappear.

Enlarged amoebae containing red blood cells, resulting from the technique described, yield very pretty specimens for class work and give students a better

[1] From the laboratories of the Army Medical School, Washington, D. C.

[2] J. H. St. John, American Journal of Tropical Medicine, 6, 319. 1926.

[3] W. C. Boeck and J. Drbohlav, *Proc. Natl. Acad. Sci.* no. 5, Washington, 1925; *Amer. Jour. Trop. Hyg.*, 5, 371.

[4] Colonel Charles F. Craig, Medical Corps, U. S. Army.

idea of *Endamoeba histolytica,* as found in infections, than is conveyed by the use of ordinary cultures of this parasite.

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PHOTOGRAPHING ANIMALS

THE scientific photographer often has trouble getting animals in a suitable position. Small animals are very active and will not stay long enough in a pose to be photographed successfully. We tried out a method for quieting snakes and lizards and it gave good results.

The photographs were made at night with Eastman flash paper, and the animals remained still in spite of the flash. The animal to be photographed was placed under an inverted box for a minute or two until all signs of commotion had ceased. The box was lifted quickly but smoothly, the flash-paper was ignited and the film was exposed. This method was also tried in broad daylight with lizards, snakes and rats, and gave good results with all the animals tried. This simple method may be good when the animal has to be moved to certain surroundings and resents it. Evidently the swift change from total darkness to a sudden glare leaves the animal dazed for a moment and gives time for the exposure.

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GEO. H. HANLEY

SPECIAL ARTICLES

ACCELERATED INFECTION IN EXPERIMENTAL POLIOMYELITIS

THE 1931 epidemic of poliomyelitis will enable investigators to study by experimental means, in monkeys, many aspects of the disease, as well as the virus inducing it. The establishment of strains of the human virus in monkeys is attended by initial difficulties which it is desirable to overcome. The experience of the past indicates that a proportion only of human strains can be implanted on the monkey. *Macacus rhesus* is the species which has been commonly employed for inoculation. It has not infrequently happened that after the first successful inoculation of monkeys with human spinal cord or medulla obtained from fatal cases of poliomyelitis, the succeeding inoculation of the spinal cord of the affected monkey has failed to induce disease. The reason for this disparity is not known. It is supposed that degeneration or virus metabolic products contained in the human cord act to make the originally inoculated monkey more susceptible to infection.

A way has been found to increase the proportion of successful inoculations of affected human and monkey spinal cords and brain stems. A number of years ago, Amoss and I observed that an attenuated strain of the monkey virus, unsuccessful on first inoculation, could be made to induce infection by repetition of the injection. We have recently employed this method in implanting 1931 human strains of virus on *Macacus rhesus* monkeys. The method consists in injecting intracerebrally and intraperitoneally, under ether anesthesia, 10 per cent. suspensions of glycerolated spinal cord. The suspensions should be free from bacteria as shown by aerobic plate tests. In our experience thus far, symptoms have either not appeared at all in from 7 to 10 days, or initial symptoms, slight in degree, have arisen and have failed to progress or have disappeared. The effects, if any occurred, tended therefore to the production of the abortive form of experimental poliomyelitis.

Time was allowed to elapse in order to determine whether the symptoms would progress or recede. As no increase occurred, reinoculation was resorted to with material from the same subjects as was employed for the original injection. Again the double—intracerebral and intraperitoneal—inoculations were made, using of course the opposite side of the brain. The symptoms which were stationary or receding were rapidly augmented; and about three days after the second injection the symptoms became pronounced, progressing quickly to paralysis and prostration, as is the rule with infected monkeys.

Not only can the abortive be converted into the progressive paralytic disease by means of reinoculation, but monkeys which develop no detectable symptoms in 11 or 12 days have been successfully infected through the employment of a second injection. The critical period seems to be about three days after the second injection. Within this brief period an accelerated reaction occurs. Whether the acceleration is due to virus alone, or in part to the alien (human) tissue elements, is not known. It may be merely a summation of virus effects, such as Amoss and I observed with monkey strains of virus. The results as described are not invariable. In one or two instances the accelerated effect either failed to arise or was delayed.

The tests to determine whether the reinoculation method suffices to establish durably in monkeys many strains of human poliomyelitis virus have yet to be completed. It remains also to be seen whether highly potent virus strains adapted to monkeys can be readily secured in this manner.

The histological changes present in the spinal cord