of human brain metabolism by means of the technique of puncture of the internal jugular vein described by Myerson, Halloran and Hirsch.[5] It has been amply demonstrated by all these methods that glucose is the main metabolic substrate for brain oxidations. In the absence of this substrate, during hypoglycemic insulin shock, it has been shown that the metabolism of the brain is diminished. This is indicated by an extremely low oxygen uptake of the brain,[6,7] in association with characteristic changes of the cortical brain potentials[8] and the onset of clinical coma. Earlier animal experiments[9,10,11,12,13,14] by numerous workers on the effect of various substrates on hypoglycemic symptoms probably have a significant bearing on brain metabolism, but the latter has not heretofore been studied directly under these conditions.

We have undertaken to study the relative availability of various substrates for brain metabolism by administering these substances intravenously during therapeutic insulin shock. We observed the effect of the various substrates on the clinical state of the patient. Simultaneously, the oxygen, glucose and lactic acid uptake of the brain was estimated from analyses of the arterial and internal jugular blood samples for these constituents. With the qualification that the observations were made on subjects suffering from schizophrenia, our data thus far reveal that glucose is readily available to the human brain as a source of energy, that lactic acid is not metabolized to any significant degree and that pyruvic acid and alcohol are not metabolized at all. It may be remarked that except for glucose these findings are in contrast to those reported on surviving brain tissue in the Warburg apparatus.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

EGGSHELL CAP METHOD OF INCUBATING CHICK EMBRYOS

AT the demonstration section of the American Society of Zoologists during the Columbus meetings of the American Association for the Advancement of Science, the authors presented a demonstration of chick embryos visible through openings in their shells, which during incubation were kept covered by eggshell caps. Embryos of 3, 6 and 9 days incubation were displayed. It will be a matter of interest to those who saw this demonstration that one of the embryos displayed subsequently hatched, as a normal chick, on January 11, after 21 days of incubation.

Similar hatching of chicks with this method in at least six different trials during the past year in this laboratory demonstrates the normalcy of development.

Various methods have been used (Kuo,[1] Orr and Windle,[2] Paff,[3] *et altera*) to observe and manipulate living chick embryos over a period of a few days, *in ovo*. However, none of the authors above reports attempts to carry embryos through to hatching. Paff recently used eggshell caps, but he reports their use only on 2-day incubated eggs as a means of introducing colchicine and incubating such embryos 24 hours or less. The present authors have tried the above-mentioned and several other methods. Normal development, complete incubation and successful hatching have resulted for them only when the eggshell cap method was used.

This method involves the removal of the eggshell and both eggshell membranes, to expose the embryo and its extra-embryonic membranes directly beneath. The embryo may thus be observed directly and is subject to manual manipulation.

The method of preparation is simple: First, an eggshell cap is prepared by breaking an egg at the small end, emptying the contents and, with scissors, cutting the empty shell around its middle. The result is approximately a half eggshell with a cross-section similar to Fig. A. It is then dried and both shell membranes are removed, as in Fig. B. The eggshell cap taken from the large end of the egg consists then of the shell only. It is sterilized with alcohol, dried, and kept in a covered container until used.

Eggs to be incubated are opened prior to incubation. Holding the egg vertically, with the large end uppermost near a bright lamp, a light area marks

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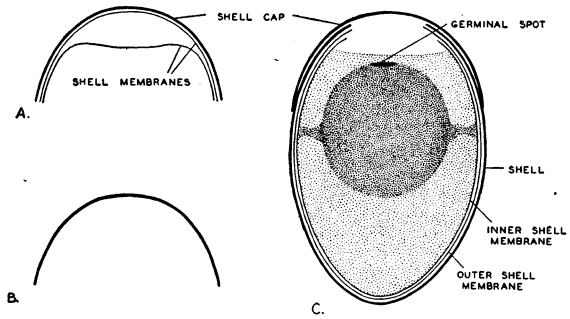

FIGS. A, B and C

the limits of the air cell in that region. After cleaning the eggshell with cotton moistened with alcohol, the outline of the air cell is marked with pencil. With tweezers a small opening is made above the air cell, and by careful chipping, pieces of shell and membrane are removed to make a circular opening somewhat smaller in diameter than the air cell beneath. With sterile tweezers the inner shell membrane is next removed to the same extent, leaving the albumen exposed. (See Fig. C.) The previously prepared shell cap is now placed over the opening and the egg is ready for incubation. The shell cap is not sealed in any way.

After several days of incubation the level of albumen within has dropped and permits the further removal of shell. Care must be taken to avoid injuring the allantois. With a relatively large opening, later stages of development are clearly visible over the entire upper surface.

In the incubator such eggs must be held vertically. Holes may be made in strips of coarse screening to hold the eggs in a standard incubator tray. If the egg is permitted to roll over on its side, albumen will be lost. Held vertically, apparently no turning of the egg is necessary. Difficulty of hatching may be avoided by sprinkling the 18-day egg with water.

The incidence of infection of embryos has been extremely low, considering the fact that the shell cap is simply laid over the eggshell opening and may be removed daily for observation of the embryo. The overlapping margin of the eggshell and its cap is apparently an effective barrier to bacteria. The porous shell of the cap apparently permits adequate gaseous exchange between the living embryo and the exterior, yet prevents excessive desiccation.

The simplicity of technique involved and the low mortality of embryos recommend this method for use in classroom demonstrations and as a source of normal living embryos readily accessible to the research worker.

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A SUPPLEMENTARY METHOD FOR THE STUDY OF ARACHNO-PIA

EXAMINATION of the leptomeninges in embedded and stained sections of brain and cord, at best, gives only a limited idea, even when made in series, of the relations and complexity of the varied and delicate parts of these membranes.

It is possible, by floating fragments of fresh or formalin-fixed tissue from water onto a glass slide, adding a drop of glycerine to the surface of the specimen and covering the whole in the usual way, with light pressure on the cover glass, to secure a preparation which can be examined with the dark field.

This gives a more or less stereoscopic picture of all the elements which are present in the tissue, as they appear free from the distortions of routine technic. When suitably prepared, very complete detail is visible. The vascular arrangement is made especially evident, and both intravascular conditions and perivascular spaces and tissues are clearly defined.

The method is of particular value for the study of meningeal concretions as well as of perivascular reactions. When combined with the routine staining technic, which brings out the detail of individual cell structures, this simple method furnishes a valuable supplement, and affords a completeness of examination which can be secured in no other way.

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