



FIGS. 1-3

by the leaf and expressed in milligrams per sq. cm is computed directly from the number of counts per minute. The second leaf from the bottom, 2 inches from the base and 6 inches from the solution, was taken for study. Five minutes after radioactive phosphorus was added it could be detected in the leaves. Curves I, II and III show that for the concentrations tested the uptake increases with the amount supplied. Curve IV shows less uptake than Curve II, because in this case the plant was allowed to stand for 24 hours in a standard nutrient solution containing 0.0008 g of phosphorus per cc prior to the addition of radioactive phosphorus. Curve A in Fig. 3 is a corresponding uptake curve for sodium.

The distribution of phosphorus in the plant changes appreciably with time. During the initial stages of the experiment the number of disintegrations was from 25 to 50 per cent. more intense for the bottom than for the top leaf; it also decreased rapidly from the base to the tip of the individual leaves. The final distribution, in general, was quite uniform between the leaves as well as along each leaf except at the tip, which remained low.

Effect of the extent of root system on uptake was investigated in detail. For both sodium and phosphorus the rate and equilibrium value were found to be the same for intact roots, for plants with roots excised at different positions and for leaves removed at the base. Plants stunted in their early growth by a deficiency in their diet took up very little sodium or phosphorus due to an impairment which began in

the root system and gradually extended up the stalk; in all stunted plants tested normal uptake was observed when the impaired section was removed.

Effect of surrounding conditions on the uptake of phosphorus is shown in Fig. 2. In each instance the plant was kept in the condition designated for 15 hours before adding radioactive phosphorus. Unfortunately it was necessary to expose the plants to room conditions for about three minutes every hour while the observations were being made. Absorption was very low while plants were in the dark but increased rapidly upon exposure to light. Low temperatures and an atmosphere of carbon dioxide decreased the uptake.

Elimination of phosphorus and sodium from the plant presents a different picture. Curve B, Fig. 3, shows decrease of sodium in the leaf when the plant (root system intact) was transferred from a solution containing radioactive sodium to one containing sodium. Curve C shows the increase of sodium in the solution due to back diffusion from the plant. Analogous results have been obtained by Jenny, Overstreet and Ayers<sup>2</sup> on the depletion of sodium from barley plants. The phosphorus content of the leaf, in contrast, decreased only slightly with time, while the radioactivity of the solution increased slowly through loss of phosphorus from the roots and lower portion of the stalk.

The writers are indebted to Dr. L. A. DuBridge, of the University of Rochester, for the radioactive phosphorus and Dr. M. A. Tuve, of the Carnegie Institution of Washington, for the radioactive sodium.

A. KEITH BREWER

ARTHUR BRAMLEY

U. S. DEPARTMENT OF AGRICULTURE

#### A METHOD OF STUDYING THE AVAILABILITY OF VARIOUS SUBSTRATES FOR HUMAN BRAIN METABOLISM DURING THERAPEUTIC INSULIN SHOCK<sup>1</sup>

PERFUSION of whole organs,<sup>2</sup> arterial and venous blood sampling from intact organs in the body<sup>3</sup> and studies of surviving excised tissues in the manometric apparatus<sup>4</sup> have been the chief methods by which the metabolism of various organs, particularly the brain, have thus far been studied. The method of blood sampling has been successfully applied to the study

<sup>2</sup> Jenny, Overstreet and Ayers, *Soil Science*, 48: 9, 1939.

<sup>1</sup> From the Division of Psychiatry, Bellevue Hospital, and the Department of Psychiatry, New York University Medical College, New York, N. Y.

<sup>2</sup> A. L. Chute and D. H. Smyth, *Quart. Jour. Exp. Med.*, 29: 379, 1939.

<sup>3</sup> E. S. London, N. P. Kotscheref, A. M. Dubinsky and A. S. Katzwa, *Arch. ges. Physiol.*, 233: 160, 1933.

<sup>4</sup> I. H. Page, "Chemistry of the Brain," Chas. C. Thomas, Baltimore, 1937.

of human brain metabolism by means of the technique of puncture of the internal jugular vein described by Myerson, Halloran and Hirsch.<sup>5</sup> 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,<sup>6,7</sup> in association with characteristic changes of the cortical brain potentials<sup>8</sup> and the onset of clinical coma. Earlier animal experiments<sup>9,10,11,12,13,14</sup> 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.

JOSEPH WORTIS

WALTER GOLDFARB

BELLEVUE HOSPITAL,  
NEW YORK, N. Y.

## 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,<sup>1</sup> Orr and Windle,<sup>2</sup> Paff,<sup>3</sup> *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

<sup>5</sup> A. Myerson, R. D. Halloran and H. L. Hirsch, *Arch. Neurol. and Psychiat.*, 17: 807, 1927.

<sup>6</sup> A. Myerson and R. D. Halloran, *Ibid.*, 33: 1, 1935.

<sup>7</sup> H. E. Himwich, K. M. Bowman, J. Wortis and J. F. Fazekas, *Jour. Nerv. and Ment. Dis.*, 89: 273, 1939.

<sup>8</sup> H. Hoagland, D. E. Cameron and M. A. Rubin, *Am. Jour. Psychiat.*, 94: 183, 1937.

<sup>9</sup> F. C. Mann and T. B. Magath, *Arch. Int. Med.*, 30: 171, 1922.

<sup>10</sup> E. C. Noble and J. Macleod, *Am. Jour. Physiol.*, 64: 547, 1923.

<sup>11</sup> P. T. Herring, J. C. Irvine and J. J. R. Macleod, *Biochem. Jour.*, 18: 1023, 1924.

<sup>12</sup> C. Voegtlin, E. R. Dann and J. W. Thompson, *Am. Jour. Physiol.*, 71: 574, 1925.

<sup>13</sup> J. A. Hewitt and H. G. Reeves, *Lancet*, 211: 703, 1926.

<sup>14</sup> S. Maddock, J. E. Hawkins, Jr., and E. Holmes, *Am. Jour. Physiol.*, 125: 551, 1939.

<sup>1</sup> Z. Y. Kuo, *Jour. Exp. Zool.*, 61: 395, 1932; *Jour. Comp. Neurol.*, 70: 437, 1939.

<sup>2</sup> D. W. Orr and W. F. Windle, *Jour. Comp. Neurol.*, 60: 271, 1934.

<sup>3</sup> G. H. Paff, *Amer. Jour. Anat.*, 64: 331, 1939.