BIOCHEMISTRY OF THE NERVOUS SYSTEM.

By Derek Richter, M.A., B.Sc.Oxon., Ph.D.Munich,

Holder of Rockefeller Research Grant, Central Pathological Laboratory and Mill Hill Emergency Hospital, London, N.W. 7.

Biochemical research has continued in spite of the war and considerable advances have been reported during the period under review. While the task of applying this new biochemical knowledge in the field of psychiatry has hardly yet begun, the underlying chemical mechanisms concerned in nervous activity are becoming considerably clearer and a few beginnings in this direction have been made.

The greatest advance in this field in the last few years has probably been in the elucidation of the phosphorylating mechanisms concerned in the metabolism of the brain. The recent isolation of a new phosphatide which is the chief component of cephalin marks a further advance in lipid biochemistry. In the present review an attempt has been made to report the main developments in the field rather than to review exhaustively every significant publication.

Carbohydrate Metabolism.

The chief fuel supplying the energy requirements of the brain is glucose. Three different things may happen to the glucose taken up from the blood by the brain: it may be (*a*) stored as glycogen, (*b*) glycolysed and eliminated from the brain as lactate, or (*c*) oxidized completely to carbon dioxide. In each case the first step is the phosphorylation of glucose to form glucose phosphate.

Phosphorylation of glucose.—When cell-free tissue extracts or homogenized brain tissue are incubated aerobically with glucose and inorganic phosphate, the glucose and phosphate disappear, and in the presence of fluoride phosphorylated products accumulate. These products include fructose diphosphate, which predominates, phosphoglyceric acid, and glucose-6-phosphate, which is believed to be the primary product (Colowick, Welch and Cori, 1940). Essential components of the system are (*a*) an oxidizable substrate, such as citrate, glutamate or succinate, (*b*) adenylic acid, (*c*) cozymase, and (*d*) magnesium ions. The adenylic acid acts as a phosphate carrier, while the oxidizable substrate is needed to provide energy for the reaction. Pyruvic acid can serve as an oxidizable substrate provided that a small amount of fumaric acid is also added. In the absence of fluoride, glucose is oxidized by brain dispersions and only small amounts of phosphorylated products accumulate.

The aerobic phosphorylation of glucose is important because it is the primary reaction in the utilization of glucose as a source of energy. The working out of the mechanism of this reaction shows how a phosphorylation may be coupled

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

with an energy-producing oxidation reaction, and it illustrates the way in which oxidative energy is utilized in the cell.

Glycogen synthesis.—Cori and Cori (1940) have reported the synthesis of glycogen *in vitro* from glucose-1-phosphate by a purified enzyme phosphorylase from muscle, brain, heart and liver. Adenylic acid is required, and the activity of the phosphorylase is increased by the addition of glutathione. The reaction is reversible, the enzyme catalysing either the synthesis or the breakdown of glycogen ; the position of the equilibrium is determined by the pH.

The synthesis of glycogen *in vitro* represents a big advance in carbohydrate biochemistry. It is of interest that the enzymes prepared from different tissues, though similar, are apparently not identical. Thus the phosphorylase from brain formed a polysaccharide giving with iodine the brown coloration typical of glycogen, while the muscle phosphorylase formed a product which gave with iodine a blue colour similar to that with starch.

The origin of the glucose-1-phosphate which is needed for the synthesis of glycogen was until recently a mystery, for the product isolated when glucose is phosphorylated directly is always the glucose-6-phosphate. Cori and his collaborators have now made this clear by showing that the enzyme phosphoglucomutase catalyses reversibly the interconversion of glucose-1- and glucose-6-phosphates (Sutherland, Colowick and Cori, 1941).

The enzyme phosphorylase which catalyses the synthesis of glycogen has been isolated as an adenylic acid complex in crystalline form (Green, Cori and Cori, 1942). The phosphoglucomutase was also separated from the other enzymes concerned in glycogen synthesis and breakdown. Glycogen has been isolated in the pure state from fresh brain (Kerr, 1938).

Glycolysis.—Brain has been considered by many investigators to be a tissue in which non-phosphorylating glycolysis occurs, but recent work has shown that it has probably a phosphorylating mechanism similar to that in muscle. Geiger (1940) showed that in concentrated extracts of brain an inhibitor is present which inhibits glycolysis, but this inhibitor is no longer active on dilution. The diluted extracts give a rapid lactic acid formation from glucose, mannose and fructose ; they also form lactic acid from glycogen. The glycolysis will take place only in the presence of inorganic phosphate, adenosine triphosphate, cozymase and magnesium ions. In the presence of fluoride, phosphoglyceric acid accumulates ; hexose diphosphate accumulates in the presence of iodoacetate. There is thus good evidence that glycolysis in brain may take a similar course to the Embden-Meyerhof scheme for muscle.

Ochoa (1941) found that hexose monophosphate and diphosphate are as readily glycolysed as glucose by cell-free preparations of brain. This again supports the view that glycolysis in brain proceeds by a phosphorylating mechanism.

Glucose and pyruvate oxidation.—The main path of carbohydrate breakdown is believed to occur through the oxidation of pyruvic acid. The formation of pyruvate from glucose is given by the Embden-Meyerhof scheme. In glycolysing cells pyruvate competes with oxygen as a hydrogen acceptor and is reduced to lactate, but in non-glycolysing cells pyruvate is oxidized to carbon dioxide.

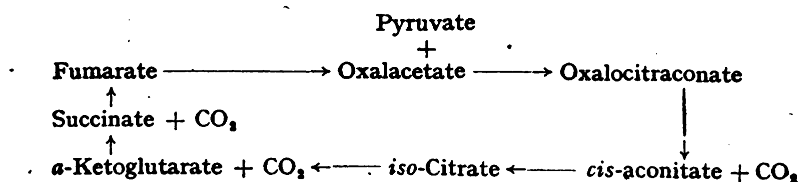
https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

Pyruvic acid is able to react in a number of different ways, and Stern (1940) has tabulated 16 different possibilities of pyruvate utilization in the living cell; any one of these mechanisms might operate under suitable conditions, depending on such factors as the concentration of reactants, permeability factors, vitamin B_1 level and pH.

The decarboxylation of pyruvic acid to give acetaldehyde and carbon dioxide is catalysed by an enzyme that may be prepared from a variety of tissues, including muscle and brain (Green *et al.*, 1941). Carbon dioxide is evolved both anaerobically and aerobically. Under suitable conditions the acetaldehyde condenses with unchanged pyruvic acid to give acetylmethyl carbinol and a second molecule of carbon dioxide. The enzyme requires diphosphothiamin (phosphorylated vitamin B_1) and the presence of magnesium ions as coenzymes.

Particular interest has been taken in the last few years in the "citric acid cycle" of Krebs (1940); this gives a possible mechanism for the oxidation of pyruvic acid by a cyclical series of reactions involving the intermediary condensation of pyruvic acid with a C_4 dicarboxylic acid to give a C_7 acid, and subsequent oxidation of this C_7 acid in successive stages to give carbon dioxide with the regeneration of the original C_4 dicarboxylic acid again.

Wood *et al.* (1942) and Evans and Slotin (1941) have recently tested the citric acid cycle by the method of studying the fate of molecules which had been previously tagged with isotopic carbon (C^{13}). Their work showed conclusively that citrate cannot be an intermediary in the oxidation of pyruvic acid, and Wood *et al.* suggested a modified cycle which, with further modifications, has now been accepted by Krebs (1942). According to the present scheme pyruvate condenses with *enol*-oxalacetate to form oxalocitraconic acid; this is oxidized with loss of CO_2 to give successively *cis*-aconitate, isocitrate, *a*-ketoglutarate, succinate, fumarate, and finally *enol*-oxalacetate again. The formation of citric acid is due to a side-reaction.



Since citric acid is no longer included in the scheme it can no longer be called the "citric acid cycle" but no new name has hitherto been proposed.

The action of malonate, which inhibits the cycle at the succinate stage, can be used for testing whether the cycle is operating in any particular tissue. Banga *et al.* (1939) demonstrated the inhibition by malonate of pyruvate oxidation by dispersed brain tissue. This gave evidence that in brain, as in muscle and other tissues, a form of "citric acid cycle" may play a part in the oxidation of carbohydrate.

Pyruvate Metabclism and Vitamin B_1.

Recent work has confirmed the view that vitamin B_1 is concerned in the utilization of pyruvate in the living tissues. The phosphorylated product

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

diphosphothiamin or cocarboxylase is probably the form in which the vitamin is effective. Guzman Barron *et al.* (1941) showed that the oxidation of a-ketoglutarate, a component of the " citric acid cycle " by avitaminotic tissues, is also accelerated by the addition of phosphorylated thiamin.

A variety of animal experiments have shown that thiamin deficiency causes an increase of pyruvic acid in the blood and urine; the pyruvate level is increased by increasing the carbohydrate intake in the diet. These experiments link up with the clinical observation of a raised pyruvate (or bisulphite binding substance) level in the blood in vitamin B_1 deficiency in man.

Bueding *et al.* (1942) found a slight but significant increase in the blood pyruvate of healthy, well nourished men within an hour after taking glucose. Vitamin B_1-deficient subjects had a higher resting level and showed a much higher rise after taking glucose. The resting pyruvate level was also raised in cases of " acute peripheral neuropathy." The apparent correlation between the blood pyruvate and thiamin has led to the idea of using the blood bisulphite binding substance (B.B.S.) as an index of vitamin B_1 deficiency; but Robinson *et al.* (1940) report that the B.B.S. is not consistently elevated in B_1 deficiency, and it may be normal in cases showing vitamin B_1 deficiency by clinical signs and by excretion tests. The B.B.S. elevation is less sensitive and less specific than the clinical tests already in use.

R. A. Peters (1940) and members of the Oxford school claim that the nervous symptoms in vitamin B_1 deficiency are directly due to the failure of the enzymes concerned in carbohydrate metabolism in the brain. This view has recently been challenged by workers at Cambridge (Banerji and Yudkin, 1942), who consider that the nervous symptoms are a secondary result of intoxication through the accumulation in the body of metabolic products associated with the defective or perverted carbohydrate metabolism. In support of their view they point out that the correlation between the metabolic defect and nervous symptoms is not precise; either may be present without the other. They have further succeeded in showing that rats will grow and thrive entirely free of symptoms on a vitamin B_1-free diet of fat and protein. Although the brain enzymes are defective, no nervous symptoms appear until they are given carbohydrate.

It has been generally considered that alcohol accelerates the onset of neuritic symptoms in alcoholic patients. Workers at the U.S. National Institute of Health have recently attempted to obtain further information on this point by means of animal experiments (Lowry *et al.*, 1942). Two series of rats were fed a thiamin-deficient diet and given either water or whisky *ad lib.* Contrary to expectations the neuropathy appeared in every case much earlier in the rats on water than in those on whisky. These experiments confirm the view that vitamin B_1 is not required in the utilization of alcohol.

Carbohydrate Utilization by the Brain.

Gibbs *et al.* (1942) have reported a valuable series of analyses of the chemical constituents of arterial and internal jugular vein blood in 50 normal male subjects. Their experiments confirm the high metabolic activity of the brain. From the oxygen uptake and glucose consumption they calculate that glucose

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

is the principal source of energy, but the glucose is not all oxidized; some of it is converted into lactic acid. New experiments of Wortis (1941) have confirmed the view that the utilization of lactate by the brain is at the most very small. They found that the injection of lactate into hypoglycaemic patients during insulin coma caused only a slight increase in the oxygen consumption of the brain and, unlike glucose, it did not support the brain oxidations sufficiently to rouse the patients from their coma. Injected lactate did not prevent or alleviate insulin convulsions in seven hepatectomized dogs.

A number of investigators have obtained evidence of abnormalities in the carbohydrate metabolism in different types of mental disease. Lennox *et al.* (1940) measured the oxygen uptake by the brain and the glucose utilization in 18 patients with *petit mal*. They found that the ratio of the glucose used to the oxygen uptake was 1·08 for the patients with *petit mal*, and 1·48 for normal individuals. This suggested that the glucose metabolism is abnormal in the brains of patients with *petit mal*. Abnormalities in the carbohydrate metabolism have also been reported in mongoloid idiots (Himwich and Fazekas, 1940).

Lipid Metabolism.

Phospholipids.—The phospholipids are generally classified under three main molecular types: the lecithins, the cephalins and the sphingomyelins. In view of their physical properties the usual criteria of chemical purity are difficult to apply to these compounds, and their separation has always been a problem of some difficulty.

Folch (1942) has recently shown that the "cephalin" of brain can be separated into three fractions which exhibit different solubilities in chloroform alcohol mixtures: fraction (*a*) has the composition formerly attributed to the whole cephalin and may be called *phosphatidyl ethanolamine*, fraction (*b*) contains serine and is *phosphatidyl serine*, and fraction (*c*) is a mixture of phospholipids, one or more of which contain inositol as a constituent.

Phosphatidyl serine and the inositol-containing fraction are much more acidic in character than phosphatidyl ethanolamine and they are isolated from brain as their potassium or sodium salts. Treatment with acid removes the alkali cations. Both the amino and the carboxyl groups of the serine component of the phosphatidyl serine molecule are free. This new phosphatide comprises between 40 and 70 per cent. of the cephalin fraction of cattle brain. In view of this discovery much of the older work on cephalin will need to be reconsidered, and it offers possibilities for new work of greater precision in this field.

Glycolipids.—Klenk (1941) has isolated a new lipid with a high sugar content from the brain of a case of Tay-Sachs' disease. It was obtained also from normal brain in a yield corresponding to 10 to 14 per cent. of the total cerebrosides. This glycolipid yielded on hydrolysis 23·6 per cent. galactose in addition to phosphate, stearic acid, sphingosine, and a new unstable amino-acid which was designated neuraminic acid.

Lipid synthesis and turnover.—A new approach to the study of lipid metabolism has been made by the method of administering organic compounds

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

which have been "tagged" by incorporating into the molecule heavy hydrogen (deuterium), isotopic carbon (C^{13}), heavy nitrogen (N^{15}), or radio-active phosphorus (P^{32}).

These new methods have enabled the classical view of the relative stability of the lipids of the brain to be confirmed with greater certainty; but they have shown that the old distinction between the metabolic and the non-metabolic or fixed lipids of the tissues can no longer be sharply maintained.

Sperry and his collaborators (1941) used the method of feeding linseed oil which has been labelled by partial reduction with deuterium. They found that appreciable amounts of deuterated lipid appeared in the liver, intestine and depot fats after feeding to rats, but only traces could be isolated from the brain. In the course of a week 20 per cent. of the fatty acids of the brain were replaced. During myelination the unsaponifiable lipids and fatty acids were rapidly replaced in young rats 15 to 19 days old. They concluded from their experiments that growth is more important than myelination in affecting the rate of deposition of brain lipids.

Hevesy and Hahn (1940) and others have taken the investigation a step further by following the rate of assimilation of radio-active phosphorus into the different lecithin, cephalin and sphingomyelin fractions. Fries and Chaikoff (1941) studied the rate of deposition of radio-active phosphorus in various parts of the brain in rats of various age groups.

An important application of the radioactive indicator technique is the demonstration with P^{32} of the synthesis of phospholipids by excised nerve and brain slices *in vitro*. The synthesis of phospholipid requires the presence of oxygen, and it is accelerated by glucose but not by pentose; it is inhibited by cyanide and other respiratory inhibitors (Fries, Schachner and Chaikoff, 1942). These experiments show that the phospholipids of brain and nerve are synthesized in the tissues themselves and are not derived from the plasma.

Distribution of lipids in the brain.—Figures giving the distribution of lipids, cholesterol and other compounds in different parts of the brain have been given by Randall (1938). Differences in the lipid content of the brains of male, female, castrate and ovariectomized rats were observed by Weil (1941).

NITROGEN METABOLISM.

Gjessing (1939) has continued his outstanding work on the nitrogen metabolism in periodic catatonia, and further cases have been reported by Hardwick and Stokes (1941) in which phasic changes in the mental state are associated with phasic changes in the nitrogen excretion. They observed in one case that increasing or diminishing the protein in the diet did not disturb the rhythm of the mental disorder. They concluded that the variation in nitrogen excretion is not secondary to the mental disturbance, nor is the mental disturbance immediately caused by the specific metabolic change. Both the metabolic and the mental changes may result from a primary cyclical change in the endocrine balance.

Greving (1941) carried out serial metabolic studies in which he compared two schizophrenics with two recovered schizophrenics maintained under the same conditions. Metabolic disturbances characterized by nitrogen retention,

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

disturbed urea synthesis and impaired cholesterol esterification preceded the phases of mental disturbance.

Gildea *et al.* (1940) have estimated the serum protein, non-protein nitrogen and lipids in the blood of patients with schizophrenic and manic-depressive psychoses. Gjessing adopted as a working hypothesis the view that a toxic substance, which may be an amine, is produced at the time of change in the nitrogen balance, and this has renewed interest in the question of toxic amines.

Amine inactivation.—The amine oxidase is the system mainly responsible for the detoxication of amines in the body; it is present chiefly in the liver, kidney and intestine, but smaller amounts are also present in other organs including the brain (Richter, 1938; Bhagvat *et al.*, 1939; Mann and Quastel, 1940). Bhagvat *et al.* (1939) have completed a study of the oxidation of 82 different amines by the amine oxidase and a good deal is now known about the specificity of the enzyme; it readily oxidizes most amines of the type $R{-}NH_2$, but it has no action on and is inhibited by isopropylamine derivatives, such as ephedrine and benzedrine. Most amines are rapidly inactivated in the body after administration by mouth; but amines such as ephedrine, mescaline, benzedrine and methylisomyn, which are not oxidized by the amine oxidase, have a much more prolonged action in the body and they are finally excreted unchanged in the urine (Richter, 1938). Phenolic amines such as epinine and corbasil are inactivated to a considerable extent by esterification (1940), and the body contains a special diamine oxidase which inactivates the diamines such as putrescine and cadaverine.

Mann and Quastel (1940) have shown that the inhibitory action of amines such as tyramine on glucose oxidation by brain slices is due in part to aldehydes, which are formed secondarily from the amines through oxidation by the amine oxidase. Benzedrine competes with other amines for the amine oxidase and it can thereby diminish the formation of aldehydes. They believe that the action of benzedrine *in vivo* may be linked with its ability to compete with amines giving rise in the body to toxic substances. The presence of such toxic substances under the conditions in which benzedrine acts *in vivo* has not, however, been demonstrated.

A sensitive method has been described for estimating amines in the blood. The method can be used to measure the rate of detoxication of *iso*-amylamine which has been injected intravenously, and this has been suggested as a test of the amine oxidase function (Richter, Lee and Hill, 1941).

The toxic amine hypothesis has been frequently considered in relation to mental disease. Our knowledge of the bodily mechanisms for inactivating toxic amines has greatly increased, but work on the clinical side has been almost entirely negative. There are considerable difficulties in applying the toxic amine hypothesis to Gjessing's data, since during the time of mental disturbance some patients are found to be in a state of positive nitrogen balance, while in others the nitrogen balance is negative. Further, the nitrogen retention observed in his cases applies to all the protein degradation products found in the urine, which suggests that the fundamental disturbance involves the whole protein catabolism, and not merely a single intermediate metabolite which might give rise to a toxic amine. Richter and Lee (1942), who have discussed

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

the toxic amine hypothesis, found no evidence of the presence of toxic amines in the blood of 58 selected psychotics of various types, though there was a slight increase in the amino-lipid fraction in 18 schizophrenics. Pugh (1940) reported the absence of tyramine in the urine of 400 mentally defective patients. Indications have been obtained (Berkenau, 1940) of a disturbed liver function in certain types of catatonic schizophrenics, but there is no evidence that amine detoxication is specifically impaired in these patients.

ACETYLCHOLINE AND ADRENALINE.

Acetylcholine.—Loewi and Hellauer (1938) found that the ventral spinal roots and preganglionic sympathetic trunk are rich in acetylcholine, while the optic nerve contains little or none. Subsequent investigators have confirmed and extended the original observations by adding to the list of nerves that are rich and to those that are poor in acetylcholine. It is now possible to generalize by saying that the distribution of acetylcholine in the peripheral nervous system is the same as that of cholinergic fibres (motor and preganglionic sympathetic fibres), while sensory nerves contain practically none. Mixed nerves contain intermediate amounts, and post-ganglionic sympathetic nerves probably contain adrenaline (Lissak, 1939).

MacIntosh (1941) has extended the investigation into the central nervous system, where he has found again that the parts containing efferent fibres are rich in acetylcholine, while parts containing only afferent fibres have little or none. The cerebral cortex and certain nuclei and tracts in the brain are rich in acetylcholine, but other nuclei and tracts are relatively poor.

In view of these findings, Dale's biochemical classification of the peripheral nervous system into cholinergic, adrenergic and sensory components is no longer an indirect inference from pharmacological data, but an experimentally demonstrable fact. The exact function of acetylcholine is not yet clear, but the fact that nervous tissue can now be separated into different varieties differing in this way in chemical composition represents a considerable advance.

Further work by Mann *et al.* (1939) on the synthesis of acetylcholine by respiring brain slices *in vitro* has added to our knowledge of the conditions under which it may occur. Mann and Quastel (1940) have shown that although glucose accelerates the reaction, the synthesis of acetylcholine does not require the presence of thiamin, since there is no appreciable difference from normal in the synthesis of acetylcholine by brain slices from thiamin-deficient pigeons.

The work of Kahlson and MacIntosh (1939) on the perfused superior cervical ganglion is of particular interest in that it links up the biochemical work on acetylcholine synthesis with physiological work on the transmission of nervous impulses. They showed that the failure of the perfused ganglion to transmit impulses when fatigued by prolonged stimulation was paralleled by the decreasing ability of the ganglion to synthesize acetylcholine. The ganglion perfused with fluid containing only inorganic salts soon showed fatigue, while fatigue could be prevented by addition to the perfusion fluid of glucose, mannose, lactate or pyruvate. These substances also promote the synthesis of acetylcholine. Fructose, sucrose and other metabolites were ineffective.

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

Choline esterase.—The choline esterase content of the human foetus increases in parallel with the development of the neuromuscular system (Youngstrom, 1941). In the sheep embryo the choline esterase activity is higher in the spinal cord than in the brain during the period of development of the activity of the spinal centres, but later the activity in the brain centres increases and it rises rapidly before birth (Nachmansohn, 1940).

The concentration of choline esterase is much higher in the head ganglion than in the giant nerve fibre of the squid; it is present mainly at the surface (Nachmansohn and Steinbach, 1942). These observations have been cited in favour of the view that choline esterase is concerned specifically with the processes involved in neural transmission. A number of observations have been reported on the serum choline esterase activity in nervous disease (Richter and Lee, 1942). High serum esterase activities have hitherto been found in (*a*) severe anxiety, (*b*) acute depressive states, (*c*) thyrotoxicosis, and (*d*) certain "post-concussional states," while low activities have been found in (*e*) catatonic stupor, (*f*) extreme debility, and (*g*) in narcosis. These observations suggest that the high serum choline esterase activity in emotional states and in thyrotoxicosis may be attributed to the increased autonomic activity or to the increased neuromuscular activity associated with these conditions. This conclusion is supported by the observation of Randall and Jellinek (1939) that the serum choline esterase activity in schizophrenics is increased significantly by "shock" therapy with insulin, since there is evidence that sympathetic activity is increased in "shock" therapy. Richter and Croft (1942) have recently found that the serum choline esterase activity is raised by as much as 50 per cent. by vigorous muscular exercise; here again a high choline esterase activity is associated with increased autonomic and neuromuscular activity.

Biochemical investigation has shown that the choline esterases form a group of enzymes which differ considerably in their individual properties (Richter and Croft, 1942). The serum choline esterase in man is not specific for choline esters, but also hydrolyses the aliphatic esters such as methyl butyrate and tributyrin.

Acetylcholine and nervous activity.—The precise role of acetylcholine in relation to nervous activity is still a matter of dispute. Some investigators believe that acetylcholine is the specific transmitter substance concerned in the transmission of nervous impulses at all myoneural junctions and at the synapses in the central as well as in the peripheral nervous system; other investigators are unwilling to consider acetylcholine as anything more than an incidental by-product of nerve metabolism.

The acetylcholine found in the tissues does not occur there for the most part in the free form, but in a bound form in which it is apparently combined with protein and therefore inaccessible to the action of choline esterase. Free acetylcholine is liberated from the bound form by the alcohol or acid used in extracting the tissue.

Lissak (1939) has confirmed that when a cholinergic nerve trunk is immersed in a bath of saline, electrical stimulation causes free acetylcholine to pass from the nerve trunk into the bathing fluid. While this experiment emphasizes the

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

relation of acetylcholine to nervous activity, it suggests that acetylcholine is not concerned exclusively in the processes at the synapses or myoneural junctions. Acetylcholine has been reported to pass into the cerebrospinal fluid after stimulation of the afferent vagus or of the hypothalamus (Chang *et al.*, 1938), which again demonstrates that acetylcholine is concerned in nervous activity.

Clark and Raventós (1938) have made a careful study of the kinetics of nactivation of acetylcholine by choline esterase. They estimate that the time required for the inactivation of acetylcholine in muscle at concentrations below 10^{-6} is of the order of 20 to 40 seconds. No process has yet been demonstrated in any tissue that can effect the destruction of small amounts of acetylcholine in a few milliseconds, as would have to occur if acetylcholine mediated the transmission of nervous impulses at the sinapses.

Schweitzer *et al.* (1939) have attempted to obtain further evidence of the role of acetylcholine by studying the action of choline esterase inhibitors in the central nervous sytem. They found that of a large group of inhibitors some acted as central depressants while some showed excitatory action. The pharmacological action appeared to be a function of the lipid solubility of the anti-choline esterase; the central excitants were all lipid-soluble, while the depressant drugs were not. There was a close quantitative relationship between the anti-choline esterase activity *in vitro* and the action *in vivo*. While these observations support the view that acetylcholine plays a part in the central nervous system, the precise role of acetylcholine is still unknown. There can be no doubt that even at very great dilutions acetylcholine exerts a profound effect on the activity of certain types of cells. The views are widely held that acetylcholine may be concerned in the processes involved in the transmission of the nervous impulse along the nerve fibres, or that it acts primarily by modifying the responsiveness of the receptor to stimulation.

Adrenaline and sympathin.—Recent work has given further evidence of the identity of adrenaline and sympathin, the "transmitter substance" of the post-ganglionic sympathetic nerves. The output of sympathin obtained in the perfusion fluid on stimulating the sympathetic nerves to the perfused rabbit's ear can be greatly increased by the addition to the perfusion fluid of a small amount of ephedrine (Gaddum and Kwiatkowski, 1938). Ephedrine sensitizes a variety of structures, including the frog's heart and the nictitating membrane of the cat to the stimulating action of adrenergic nerves; it has the same sensitizing action on the stimulation by adrenaline.

Ephedrine inhibits the amine oxidase, which has been shown to oxidize adrenaline *in vitro*, and Gaddum has suggested that the pharmacological actions of ephedrine may be attributed to its inhibiting the amine oxidase *in vivo* and so preventing the destruction of adrenaline. This would explain the increased yield of sympathin from the rabbit's ear when ephedrine is added to the perfusion fluid, and the action of ephedrine in sensitizing structures such as the nictitating membrane to adrenaline action. According to this view the amine oxidase and ephedrine play a role corresponding to that of choline esterase and eserine. While this theory explains a great many of the facts, further work by Richter and Tingey (1939) has shown that there is no significant inhibition of the amine oxidase by ephedrine at the concentrations used by Gaddum.

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

They conclude that it is unlikely that the amine oxidase is specially concerned in the inactivation of adrenaline.

The ease with which adrenaline is oxidized *in vitro* has led it to be supposed that adrenaline is also inactivated by oxidation *in vivo*. It has now been shown that after the administration of adrenaline a considerable part of the adrenaline is excreted in the urine in the form of an adrenaline ester (Richter, 1940). Richter and MacIntosh (1941) have found that the adrenaline ester is pharmacologically inactive when tested on the blood pressure, nictitating membrane and intestine of the cat. Chemical evidence indicates that the adrenaline ester is probably a sulphate ester, in which esterification has occurred at one of the phenolic hydroxyl groups. These experiments suggest that the "sulphosynthase" system, which is responsible for the synthesis of sulphate esters, may be the system mainly concerned in the inactivation of adrenaline *in vivo*.

Lissak (1939) has shown that adrenaline is not liberated exclusively at the nerve endings, but also from the nerve trunks of adrenergic nerves when they are electrically stimulated.

Mechanism of adrenaline action.—The biochemical mechanism of the action of adrenaline on plain muscle is still obscure; but in addition to this effect adrenaline also mediates the glycogen breakdown in emotional hyperglycaemia, and some information as to the chemical mechanism of this process has now been obtained. The glycogen breakdown in the liver has generally been attributed to an activation of the liver amylase by adrenaline. It has now been shown that adrenaline acts by increasing the rate of phosphorylation of glycogen (Lee and Richter, 1940). This is not due to a direct activation of the phosphorylase, but it may be due to a coupling of the phosphorylating system with the reducing systems of the cell, which results in the rapid phosphorylation of glycogen and the breakdown of the glucose-1-phosphate to give free glucose.

The action of adrenaline in producing the subjective sensations of anxiety has interested many investigators. Dynes and Tod (1940) observed that the emotional response is absent in deteriorated schizophrenics, although they show the physiological responses to adrenaline. Thorley (1942) has confirmed the view that patients with anxiety states are more sensitive to adrenaline than normal subjects, and he has shown that they give an increased physiological response as well as an increased emotional reaction.

Respiration in the Central Nervous System.

Effects of oxygen lack.—Resistance to oxygen lack is a problem of practical importance in wartime in connection with high altitude flying. Emotional control and judgment are impaired by relatively mild degrees of anoxia, and military flying is conducted up to altitudes (40,000 ft.) so high that anoxia may occur even when inhaling 100 per cent. oxygen. Barach *et al.* (1941) have found that resistance to oxygen lack is related to the basal metabolic rate and the resistance is greatly increased in thyroidectomized animals. They conclude also that the susceptibility of pilots to "pilot error" due to anoxia may be

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

significantly increased by the mild carbon monoxide poisoning which results from cigarette smoking. In 18 inhaling smokers the arterial blood was found to have a mean carbon monoxide saturation of 5·7 per cent. after smoking 20 cigarettes between 9 a.m. and 4 p.m. Lyman *et al.* (1941) studied the effects of pseudo-ascents to high altitudes in a decompression chamber on the EEG. They observed periods of general flattening of the waves at pressures corresponding to 18,000 ft. in subjects who had removed their gas masks, but the changes were variable in constancy and degree.

The excitability of the cerebral cortex *in vivo* depends on the blood glucose level as well as on the oxygen tension. These two factors act synergistically, so that the effect of anoxia on the brain potentials is greatly aggravated by insulin hypoglycaemia (Gellhorn and Kessler, 1942), while the effect of hypoglycaemia can be offset by the inhalation of 100 per cent. oxygen. Anoxia and hypoglycaemia together depress the cortical potentials more than corresponds to the algebraic sum of the separate effects. While the somatic nervous system is depressed in anoxia, the sympathetic system is in a state of increased tonicity and excitability. There is a similar synergism between anoxia and hypoglycaemia in their effects on the sympathetic nervous system.

Hoagland *et al.* (1939) showed that thyroxin and dinitrophenol, which increase the basal metabolic rate, both accelerate α-wave frequencies, while factors such as hypoglycaemia and narcotics, which lower the metabolic rate, lower the frequencies. They conclude that the relative frequency of the cortical rhythms is determined basically by the respiratory rate of the cortical cells.

Respiration of the isolated neuron.—A considerable literature now exists on the gross respiration of the central nervous system, and evidence is accumulating on the respiration of structurally delimited regions. Pearce and Gerard (1942), in a publication that is of considerable interest, have now measured the respiratory intensity per neuron in various parts of the brain of the frog. The amounts of tissue used were of the order of 1 mgm., and the time elapsing between decapitation and commencement of respiratory measurement was as little as 10 minutes. The number of cells was measured by macerating the tissue and counting the cell nuclei in a homogeneous suspension in a solution containing acetic acid and gentian violet. The oxygen uptake per neuron varied from $9{\cdot}1 \times 10^{-6}$ cu. mm./hr. in the hippocampus to $1{\cdot}7 \times 10^{-6}$ in the cerebellum.

Nachmansohn and Steinbach (1942) have followed the lead of the electrophysiologists in utilizing the giant neurons of the squid to study the distribution of respiratory enzymes in different parts of the nerve cell. They found that the succinic dehydrogenase is concentrated in the axoplasm, which contains 90 per cent. of the total amount, and much less is present in the sheath. This is the reverse of the distribution of diphosphothiamin, which, like choline esterase, is concentrated mainly at the surface.

Intermediary oxidation systems.—The system chiefly concerned in oxidations by molecular oxygen in the brain is the cytochrome oxidase system. The dehydrogenases catalyse the transfer of hydrogen from the oxidizable metabolites of the cell to the cytochromes. The cytochrome oxidase then catalyses the oxidation of the cytochromes by molecular oxygen.

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

The cytochrome oxidase was generally believed to be a copper-protein complex. Keilin and Hartree (1939) have now described a new iron-porphyrin-protein complex "cytochrome component a_3" which they believe to be identical with the cytochrome oxidase. The component a_3 is thermolabile and gives characteristic absorption bands. It combines with carbon monoxide and the other respiratory inhibitors. The new component a_3 is apparently identical with the "respiratory enzyme" of Warburg. Narcotics, such as urethane, inhibit the reduction of components, *a*, a_3 and *c* by the dehydrogenase systems; they also inhibit the oxidation of component *b*. Work on the individual properties of the different cytochromes is actively proceeding in several laboratories and the isolation of cytochrome *a* has now been reported.

Most of the work on the cytochromes has been done with muscle tissue, but in view of the close similarity between the metabolism of muscle and of nervous tissue it can hardly be doubted that these results are applicable to brain. The presence of the cytochromes and of cytochrome oxidase has been demonstrated in brain; both are present in high concentration in the grey matter of the cortex, but relatively deficient in nerve.

Oxidation and phosphate transfer.—During the last few years interest has been focused on the relationship between oxidation reactions and phosphate transfer. Reference has been made already to the coupled aerobic phosphorylation of glucose. Endothermic phosphorylation coupled with oxidation would appear to be one of the main mechanisms for the transfer of energy used by the cell.

Ochoa (1941) has studied the coupling of phosphorylation with the oxidation of pyruvate in pigeon brain preparations. The phosphorylation of adenylic acid could be inferred from the fact that addition of catalytic amounts enabled phosphate to be transferred to other substrates. At least two molecules of phosphate were transferred for each atom of oxygen consumed in oxidizing the pyruvate. Needham *et al.* (1941) have obtained evidence that a transfer of phosphate may occur from adenosine triphosphate to protein; this was indicated by physical changes that were observed in the myosin fraction of the proteins of muscle when adenosine triphosphate was added and thereby broken down. Adenosine triphosphate has recently been isolated from brain (Kerr, 1941), and it appears likely that here again there may be an analogy between muscle and brain metabolism. Mann and Quastel (1941) have studied the breakdown of diphosphopyridine nucleotide, an important constituent of the dehydrogenase systems, by suspensions of brain. They concluded that the breakdown is due to a nucleotidase, and they found that the addition of nicotinamide prevented the breakdown. Stone (1940) has published analyses of the inorganic phosphate, phosphocreatine, adenosine triphosphate and other phosphate fractions in brain.

Narcotics and Convulsants.

Further work has been reported by Michaelis and Quastel (1941) on the inhibition of respiratory processes by narcotics. Anaerobic glycolysis is not inhibited by chloretone, but the inhibition of the oxidation of glucose, lactate

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

and pyruvate has been studied using brain slices or minced brain tissue. It is concluded that the inhibitory action of chloretone is due to its affecting either a flavoprotein or some component of the respiratory system intermediate between the flavoprotein and the cytochrome oxidase. The point at which narcotics act has been discussed also by Keilin and Hartree (1939). Fuhrman *et al.* (1941) have criticized the view that narcosis is due specifically to an inhibition of glucose oxidation in the brain. They found that phenobarbital and 1 : 3-dimethylbutylethyl-barbiturate, which is closely similar to phenobarbital in structure, both inhibit the oxidation of glucose by brain slices; but whereas the one compound is a depressant, the other is an excitant and produces convulsions.

Stone (1938) has studied the lactic acid content of the brains of normal and of narcotized mice, which were killed by immersion in liquid air. The mean lactate concentration was greatly lowered by narcotization with phenobarbital, amytal and ether. The brain lactate was increased during exercise and greatly increased during convulsions induced by picrotoxin, metrazol and sodium cyanide. During insulin convulsions the brain lactate was lowered. It would be attractive to suppose that the brain lactate level gives an index of the degree of nervous activity, corresponding to the increased lactate level during activity in muscle; but against this view is the fact that during nicotine convulsions the brain lactate level remained unchanged. The lactate rise during cyanide convulsions was also much larger than with the other convulsants, which suggests that the lactate level is determined by factors other than the degree of nervous activity. Control experiments showed that the high brain lactate values cannot be attributed to diffusion of lactate from the blood to the brain. Brain phosphocreatine increases and inorganic phosphate decreases during barbiturate anaesthesia (Stone, 1940). Stone's results are some of the first reliable data that have been obtained hitherto on the intermediary metabolism of the brain during narcosis, exercise and convulsions.

A great many papers have appeared recently on the antagonisms between the various central excitants and central depressants. These drugs show considerable individual specificity in their effects on the different centres. Rosenthal (1941) has shown, for example, that picrotoxin hyperglycaemia is regularly suppressed by many hypnotics, such as the barbiturates, chloretone and paraldehyde, which depress the blood-sugar regulating centres; but urethane even in large doses cannot annul the hyperglycaemic action of picrotoxin. Depression of the sugar-regulating centres can be obtained independently of any action on the sleep centres.

Watterson and Macdonald (1939) have found that cardiazol convulsions are inhibited by the parasympathomimetic drugs carbaminoyl-choline and acetyl-β-methylcholine and by sodium cyanide. They attribute the inhibitory action to cerebral vasodilatation, which increases the rate of cerebral blood flow.

Putnam and Merritt (1941) have discussed the various chemical factors which may affect convulsions. Seizures may be excited in predisposed individuals by hypoglycaemia, oxygen deficiency, pH shift of the blood and by numerous drugs; they may be prevented by an excess of carbon dioxide,

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

glucose, and in some cases of *grand mal* by anti-convulsants. A shift in the acidity of the milieu of the nerve cells may become a decisive factor in the production of convulsions. They suggest as a hypothesis of the mechanism of anti-convulsant action that the anti-convulsants enter the nerve cells *via* the lipid membranes and there break down to give stable aromatic acids.

The brain glycogen level falls after administering insulin, but convulsants such as strychnine, metrazol and picrotoxin do not lower the brain glycogen appreciably (Kerr and Antaki, 1938).

Mineral and Water Metabolism.

Improvements in the technique of measuring the plasma, lymph and extracellular fluid volumes have been discussed in a recent review of the literature on water metabolism by Peters (1942). The use of the Evans blue dye is a considerable improvement on the older methods for measuring the plasma volume.

Use has been made of radio-active isotopes for studying the distribution of sodium and potassium in the tissues (Noonan, Fenn and Haege, 1941). When potassium is given to an animal it is soon excreted, and the extracellular fluid is left with only the minimal amount that is required to supply the demands of the cells. Sodium and chloride, on the other hand, are largely confined to the extracellular fluids; muscle cells are practically free from sodium and chloride, but an appreciable amount of sodium is present in the cells in the central nervous system. The influence of endocrine factors on the distribution of sodium and potassium has been discussed (Peters, 1942).

It has been reported that individuals who are subjected daily to low pressures, as in flying at above 12,000 ft., excrete on the average 100 per cent. more urine than when they remain at sea level (Silvette, 1942). Rats show the same phenomenon, and they are therefore available for experimental work on this form of diuresis. In rats that are repeatedly subjected to low pressures, a hypertrophy of the kidneys, adrenal glands and lymphoid tissue can be demonstrated.

The application of deuterium oxide (heavy water) to the cerebral cortex produces a state of catalepsy in cats and monkeys. Injected deuterium oxide disappears from the cerebrospinal fluid within 30 minutes. The replacement of 10 to 22 ml. of cerebrospinal fluid by deuterium oxide saline in catatonic patients produced no appreciable change in the psychiatric or neurological picture (Stern and Dancy, 1941). A cellular reaction was observed in the fluid but there was no change in the pulse rate, temperature, respiration, blood pressure, basal metabolic rate, blood cholesterol, glucose tolerance or kidney function.

Potassium.—Potassium is believed to affect the permeability and the electrical potential of the cell membranes. The role of potassium in relation to nervous activity may be that of modifying the responsiveness of the receptor to the neural transmitting agent.

Dawes (1941) has reinvestigated the vasodilator action of potassium, and his work clears up a number of earlier contradictions in the literature. The

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

effects of potassium depend on the vascular tone, temperature and oxygenation as well as on the dose; they are complicated by the fact that potassium causes a release of adrenaline from the adrenal glands and these two principles act antagonistically. He concludes that the function of potassium is closely related to muscular work.

Thompson and Tice (1941) found that the effect of prostigmine in myasthenia gravis is related more closely to its action in liberating potassium from the muscles than to its anti-choline-esterase action. The effects of prostigmine in the dog also parallel the changes in the serum potassium rather than the inhibition of choline esterase. These experiments agree with the work of Cumings on the role of potassium in myasthenia gravis. The view that prostigmine acts in myasthenia by inhibiting the choline esterase is no longer widely held.

Water balance and epileptic seizures.—Greville *et al.* (1940) have made a careful study of the relation of water balance to seizures in epileptics. Their work is of particular interest from the point of view of experimental technique and the special precautions they took to eliminate all the possible sources of error. Their measurements, which extended over periods of up to five months, included the urine volume, body weight, insensible water loss and corrected water balance, as well as estimations of the total blood solids and the total plasma and non-protein nitrogen. They found that retention of body water did not always precede a seizure; at the most 10 seizures out of 22 were preceded by a rise in the weight curve. They concluded that water retention may be one of the factors which precipitate a seizure, or that the conditions which determine the onset of a seizure may on occasion influence the water exchange.

Stone *et al.* (1942), who used the specific gravity of the blood and serum as an index of hydration, also concluded from their experiments that water retention does not necessarily precede a fit; but relatively few of their patients gave seizures when tested by the hydration and pitressin method. Wachter (1939) obtained convulsions in 9 out of 15 epileptics after administering water and pitressin, while Blyth (1943) obtained seizures in 45 out of 49 patients by this method. The differences are most probably due in part to the different types of patients used and in part to differences in the experimental technique.

Menière's syndrome.—The labyrinthine apparatus would appear to be a particularly sensitive indicator of certain forms of metabolic disturbance, and there is evidence that a considerable proportion of cases are attributable to errors in the mineral or water metabolism. Attacks may be precipitated in certain patients by drinking fluids and, conversely, restriction of water intake may be used as a method of control. Some investigators have concluded that the serum potassium level is the most important factor, and considerable improvement has been reported from the administration of potassium by mouth. Horton (1941) believes that local oedema due to altered permeability of the capillaries of the labyrinth is the primary lesion, and he reports that treatment with histamine gave prompt relief from vertigo, nausea and vomiting in 49 consecutive cases.

Acid-base balance.—Gesell *et al.* have found that when the respiratory

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

reflex is provoked by stimulating Hering's nerve in an anaesthetized dog, the time of after-discharge, as measured by the hyperventilation, is inversely proportional to the time of stimulation (Gesell, Brassfield and Hamilton, 1942). Such an inverse relation of after-discharge to stimulation stands in marked contrast to the established findings of Sherrington, according to which reflex after-discharge is directly proportional to the amount of stimulation. They conclude that the alkalosis due to the hyperventilation is the reason for the apparent discrepancy, and they attribute the effect to the decreased stability of acetylcholine in the central nervous system at an alkaline pH. While it is unlikely, in the reviewer's opinion, that the slight increase in the rate of acetylcholine breakdown associated with the observed pH shift from 7·47 to 7·62 can account for the effect, this peculiarity of the respiratory reflex would appear to be worthy of note.

Dyspnoea is one of the most constant symptoms in "effort syndrome," and Soley and Shock (1938) have concluded that "effort syndrome" results from the washing out from the blood of carbon dioxide by hyperventilation. Jones and Scarisbrick (1941) have made careful measurements of the respiratory exchange in a large number of patients with "effort syndrome," but their results do not confirm the views of Soley and Shock. The mean respiratory rate in the patients with "effort syndrome" was almost double that of normal controls, and a number of other differences were noted; but the mean minute volume in the patients with "effort syndrome" was normal when at rest. The respiratory response to exercise was greater in the "effort syndrome" patients, however, and persisted longer than in the normal controls. Patients with "effort syndrome" do not form a homogeneous group, and evidence was obtained that in one type of patient with "effort syndrome" the nervous symptoms of exhaustion may be due to an abnormally rapid rise in the blood lactic acid during exercise (Jones and Scarisbrick, 1942).

Evidence has been accumulating of an impairment of the mechanism for the maintenance of the acid-base balance in patients with epilepsy. Favill *et al.* (1940) found that 15 epileptic patients differed from normal in that the blood pH took much longer to return to normal after a period of over-breathing. There was also no compensatory fall in the alkali reserve. Gibbs *et al.* (1940) made serial estimations of the carbon dioxide content of the arterial and internal jugular vein blood in 94 patients with epilepsy and observed an abnormality in 70 per cent. Similar findings have been reported by other investigators.

Cerebrospinal Fluid.

Recent work has provided new data on the concentrations of a number of metabolites in the cerebrospinal fluid. Work on the detection of pathological constituents has continued. Friedemann (1942) has recently reviewed the literature on the blood-brain barrier, describing particularly the work on the permeability of the barrier to drugs, viruses, toxins and dyes.

Wallace and Brodie (1940) have reinvestigated the question of the source of the cerebrospinal fluid by injecting bromide or iodide into the systemic circulation, and by following the passage of these anions into the extracellular

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

brain fluid and cerebrospinal fluid. Iodide injected intravenously reached a given concentration earlier in the extracellular brain fluid than in the cerebrospinal fluid. Bromide injected intravenously was found to enter the cerebrospinal fluid in a region of the cord that had been previously isolated by ligation from the cisterna magna. They concluded that anions are able to pass from the general circulation to the cerebrospinal fluid *via* the extracellular spaces of the brain and cord. Bernstein and Gregersen (1938) found that the two sugars, glucose and galactose, when injected into the subarachnoid space, disappeared at similar rates. Since glucose is rapidly utilized by the brain, while galactose is not, they concluded that the brain is unable to use metabolites such as glucose in the cerebrospinal fluid.

Estimations of the thiamin content of the cerebrospinal fluid by the phycomyces method have shown that up to 18 μg. per cent. may be present (Säker, 1940). The thiamin concentration in the cerebrospinal fluid is not directly related to the blood level, nor has it been shown to be related to any pathological condition. Intraspinal injection of thiamin is followed by rapid resorption into the blood from the lumbar and subarachnoid spaces, and conversely thiamin administered intravenously passes rapidly into the cerebrospinal fluid. Intraspinal injection has thus no advantage over any other route for administering thiamin.

The presence of choline esterase in the cerebrospinal fluid has been demonstrated in 23 individuals who were believed to be normal (Birkhäuser, 1941). The amount present is far lower than in the blood and varied from 4 to 24 (expressed as cu.mm. CO_2 per 0·5 ml. cerebrospinal fluid per 120 minutes) with a mean of 14. There was no connection between the choline esterase and the leucocyte count. The mean choline esterase activity in 18 schizophrenics was 19·4.

Proteins and other foreign substances inhibit the crystallization of crystalloids, and a simple test, based apparently on this principle, has been devised by Tonesco for detecting abnormal constituents in the cerebrospinal fluid. Changes in the crystal habit of sodium chloride are observed when small amounts of cerebrospinal fluid are added to a drop of 0·8 per cent. sodium chloride solution, which is evaporated on a microscope slide. The value of the test has been investigated in a variety of pathological conditions (Ström-Olsen and Kite, 1942). The test is strongly positive in general paresis. While the precise physiochemical basis of the test is obscure, it is of interest in view of its extreme sensitivity. Ford Robertson and Colquhoun (1940) have obtained new data on the mechanism of the Meinicke clarification reaction. Perelli (1941) has studied the calcium chloride flocculation test in 13 cases of acute poliomyelitis. He concludes that the test is valuable in diagnosis, and it may be useful in following the treatment.

Experimental Techniques.

One of the most promising features of recent biochemical work has been the rapid development of new experimental techniques. The chemical methods for the separation and isolation of the chemical constituents of the tissues have

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

been extended by the introduction of a wide variety of new adsorption techniques, which are now penetrating into every field of biochemistry. Specific adsorbants for the separation of basic substances such as acetylcholine and histamine, for example, are now available. There is also a noticeable increase in the delicacy of the chemical methods employed, so that substances too unstable for isolation by the older methods can now be handled satisfactorily.

Micro-analytical methods are still developing rapidly, and the literature contains a large number of new methods and new modifications of methods for estimating the more important constituents of the tissues. Ultra-violet fluorimetric methods enable vitamin B_1, for example, to be estimated in quantities of less than a millionth of a gramme. Vitamin C can be estimated in the blood. Estimations of blood iodoprotein have been used for controlling the treatment of patients with thyroid disorders. Many of the phosphorylated intermediates in carbohydrate metabolism can now be estimated, and an enzymic method based on catalysis of the breakdown of hexosediphosphate is used for estimating cozymase. Brand and Sperry (1941) have described a new method for estimating cerebrosides by hydrolysing with acid and titrating the galactose formed with ceric sulphate. These are only a few examples of a large number that might be cited.

Apart from chemical methods a number of new physical methods are finding increasing application in biochemistry. Electrophoretic methods for separating proteins may be mentioned in this connection, and also the glass electrode method for estimating the pH of the blood. The photoelectric absorptiometer has replaced the colorimeter for many purposes, and by the use of special colour filters greater selectivity can be obtained than is possible by eye. Micro-respirometric methods have improved, so that the respiration of less than 1 mgm. of tissue can now be measured.

The method of labelling molecules by the use of isotopes such as deuterium, isotopic carbon and radio-active phosphorus is an important new development. Radio-active phosphorus (P^{32}), for example, is prepared by the bombardment of P^{31} with deuterons accelerated in the cyclotron. The radio-active phosphorus can then be estimated in the presence of inactive phosphorus with the Geiger-Müller counter.

These experimental methods are still comparatively new in biochemistry, but it is clear already that some of them have important applications in the study of the nervous system and so, ultimately, will help towards solving the problems of psychiatry.

LITERATURE CITED.

Banerji, G. G., and Yudkin, J. (1942), *Biochem. J.*, **36**, 530.
Banga, I., Ochoa, S., and Peters, R. A. (1939), *ibid., J.*, **33**, 1980.
Barach, A. L., Eckman, M., and Malmont, N. (1941), *Amer. J. Med. Sci.*, **202**, 336.
Berkenau, P. (1940), *J. Ment. Sci.*, **86**, 514.
Bernstein, A. O., and Gregersen, M. I. (1938), *Amer. J. Physiol.*, **123**, 747.
Bhagvat, K., Blaschko, H., and Richter, D. (1939), *Biochem. J.*, **33**, 1338.
Birkhäuser, H. (1941), *Schweiz. Arch. f. Neur. u. Psychiat.*, **46**, 185.
Blyth, W. (1943), *Brit. Med. J.*, **1**, 100.
Brand, F. C., and Sperry, W. M. (1941), *J. Biol. Chem.*, **141**, 545.
Bueding, E., Wortis, H., Stern, M., and Esteronne, D. (1942), *J. Clin. Invest.*, **21**, 85.
Chang, H. C., Hsieh, W. M., Li, T. H., and Lim, R. K. S. (1938), *Chinese J. Physiol.*, **13**, 153.

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

CLARK, A. J., and RAVENTÓS, J. (1938), *Quart. J. Exp. Physiol.*, **28**, 155 and 177.
COLOWICK, S. P., WELCH, M. S., and CORI, C. F. (1940), *J. Biol. Chem.*, **133**, 359 and 641.
CORI, C. F. (1940), *Endocrinology*, **26**, 285.
CORI, G. T., and CORI, C. F. (1940), *J. Biol. Chem.*, **135**, 733.
DAWES, G. S. (1941), *J. Physiol.*, **99**, 224.
DYNES, J. B., and TOD, H. (1940), *J. Neurol. Psychiat.*, **3**, 1.
EVANS, E. A., and SLOTIN, L. (1941), *J. Biol. Chem.*, **141**, 439.
FAVILL, J., AMERY, L., and FREELAND, M. (1940), *Trans. Amer. Neurol. Ass.*, **66**, 74.
FOLCH, J. (1942), *J. Biol. Chem.*, **146**, 35.
FORD ROBERTSON, W. M., and COLQUHOUN, D. B. (1940), *J. Ment. Sci.*, **86**, 66.
FRIEDEMANN, U. (1942), *Physiol. Reviews*, **22**, 125.
FRIES, B. A., and CHAIKOFF, I. L. (1941), *J. Biol. Chem.*, **141**, 479.
FRIES, B. A., SCHACHNER, H., and CHAIKOFF, L. L. (1942), *ibid.*, **144**, 59.
FUHRMAN, F. A., MATIN, A. W., and DILLE, J. M. (1941), *Science*, **94**, 421.
GADDUM, J. H., and KWIATKOWSKI, H. (1938), *J. Physiol.*, **94**, 87.
GEIGER, A. (1940), *Biochem. J.*, **34**, 465.
GELLHORN, E., and KESSLER, M. (1942), *Amer. J. Physiol.*, **136**, 1.
GESELL, R., BRASSFIELD, C. R., and HAMILTON, M. A. (1942), *ibid.*, **136**, 604.
GIBBS, E. L., LENNOX, W. G., and GIBBS, F. A. (1940), *Arch. Neurol. Psychiat.*, **43**, 223.
GIBBS, E. L., LENNOX, W. G., NIMS, L. F., and GIBBS, F. A. (1942), *J. Biol. Chem.*, **144**, 325.
GILDEA, E. F., MANN, E. B., and BIACH, R. W. (1940), *Arch. Neurol. Psychiat.*, **43**, 932.
GJESSING, R. (1939), *Arch. f. Psychiat.*, **109**, 525.
GREEN, A. A., CORI, G. T., and CORI, C. F. (1942), *J. Biol. Chem.*, **142**, 447.
GREEN, D. E., WESTERFELD, W. W., VENNESLAND, B., and KNOX, W. E. (1941), *ibid.*, **140**, 683.
GREVILLE, G. D., JONES, T. S. G., and HUGHES, W. F. G. (1940), *J. Ment. Sci.*, **86**, 195.
GREVING, H. (1941), *Arch. f. Psychiat.*, **112**, 613.
GUZMAN BARRON, E. S., GOLDINGER, J. M., LIPTON, M. A., and LYMAN, C. M. (1941), *J. Biol. Chem.*, **141**, 975.
HARDWICK, S. W., and STOKES, A. B. (1941), *Proc. Roy. Soc. Med.*, **34**, 733.
HEVESY, G., and HAHN, L. (1940), *Kgl. Danske Videnskab. Selskab., Biol. Medd.*, **15**, No. 5, 1.
HIMWICH, E., and FAZEKAS, J. F. (1940), *Arch. Neurol. Psychiat.*, **44**, 1213.
HOAGLAND, H., HIMWICH, H. E., CAMPBELL, E., FAZEKAS, J. F., and HADIDIAN, Z. (1939), *J. Neurophysiol.*, **2**, 276.
HORTON, B. J. (1941), *Surg. Gynec. and Obstet.*, **72**, 417.
JONES, M. S., and SCARISBRICK, R. (1941), *Proc. Roy. Soc. Med.*, **34**, 549.
Idem (1942), *War Medicine*, **2**, 901.
KAHLSON, G., and MACINTOSH, F. C. (1939), *J. Physiol.*, **96**, 277.
KEILIN, D., and HARTREE, E. F. (1939), *Proc. Roy. Soc.*, B., **127**, 167.
KERR, S. E. (1938), *J. Biol. Chem.*, **123**, 443.
Idem (1941), *ibid.*, **140**, 77.
Idem and ANTAKI, A. (1938), *ibid.*, **122**, 49.
KLENK, E. (1941), *Z. Physiol. Chem.*, **268**, 50.
KREBS, H. (1942), *Biochem. J.*, **36**, Proc. ix.
Idem and EGGLESTON, L. V. (1940), *ibid.*, **34**, 442.
LEE, M., and RICHTER, D. (1940), *ibid.*, **34**, 353 and 551.
LENNOX, W. G., GIBBS, E. L., and GIBBS, F. A. (1940), *Trans. Amer. Neurol. Ass.*, **66**, 81.
LISSAK, K. (1939), *Amer. J. Physiol.*, **125**, 778.
Idem (1939), *ibid.*, **127**, 263.
LOEWI, O., and HELLAUER, H. (1938), *Pflüger's Arch. ges. Physiol.*, **240**, 769.
LOWRY, J. U., SCHRELL, W. H., DAFT, F. S., and ASHBURN, L. L. (1942), *J. Nutrition*, **24**, 73.
LYMAN, R. S., CARLSON, W. A., and BENSON, O. O. (1941), *J. Aviat. Med.*, **12**, 115.
MANN, P. J. G., and QUASTEL, J. H. (1940), *Nature*, **145**, 856.
Idem (1940), *Biochem. J.*, **34**, 414.
Idem (1941), *ibid.*, **35**, 502.
MANN, P. J. G., TENNENBAUM, M., and QUASTEL, J. H. (1939), *ibid.*, **33**, 822.
MACINTOSH, F. C. (1941), *J. Physiol.*, **99**, 436.
MICHAELIS, M., and QUASTEL, J. H. (1941), *Biochem. J.*, **35**, 518.
NACHMANSOHN, D. (1940), *J. Neurophysiol.*, **3**, 396.
Idem and STEINBACH, H. B. (1942), *ibid.*, **5**, 109.
NEEDHAM, J., SHEN, S. C., NEEDHAM, D. M., and LAWRENCE, A. S. C. (1941), *Nature*, **147**, 766.
NOONAN, T. R., FENN, W. O., and HAEGE, L. (1941), *Amer. J. Physiol.*, **132**, 474.
OCHOA, S. (1941), *J. Biol. Chem.*, **141**, 245.
Idem (1941), *ibid.*, **138**, 751.
PEARCE, J., and GERARD, R. W. (1942), *Amer. J. Physiol.*, **136**, 49.
PERELLI, C. (1941), *Minerva Med.*, **1**, 363.
PETERS, J. P. (1942), *Ann. Review of Physiol.*, **4**, 89.
PETERS, R. A. (1940), *Chem. and Ind.*, **59**, 373.
PUGH, C. E. M. (1940), *J. Ment. Sci.*, **86**, 244.
PUTNAM, T. J., and MERRITT, H. H. (1941), *Arch. Neur. Psychiat.*, **45**, 505.
RANDALL, L. O. (1938), *J. Biol. Chem.*, **124**, 481.

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press

Idem and JELLINEK, E. M. (1939), *Endocrinology*, **25**, 279.
RICHTER, D. (1938), *Biochem. J.*, **32**, 1763.
Idem (1940), *J. Physiol.*, **98**, 361.
Idem and CROFT, P. G. (1942), *ibid.*, **101**, 9P.
Idem (1942), *Biochem. J.*, **36**, 746.
RICHTER, D., and LEE, M. H. (1942), *J. Ment. Sci.*, **88**, 1.
Idem (1942), *ibid.*, **88**, 428 and 435.
RICHTER, D., and MACINTOSH, F. C. (1941), *Amer. J. Physiol.*, **135**, 1.
RICHTER, D., and TINGEY, A. H. (1939), *J. Physiol.*, **97**, 265.
RICHTER, D., LEE, M. H., and HILL, D. (1941), *Biochem. J.*, **35**, 1225.
ROBINSON, W. D., MELNICK, D., and FIELD, H. (1940), *J. Clin. Invest.*, **19**, 483.
ROSENTHAL, F. E. (1941), *J. Pharmacol.*, **73**, 446.
SÄKER, G. (1940), *Klin. Woch.*, **19**, 99.
SCHWEITZER, A., STEDMAN, E., and WRIGHT, S. (1939), *J. Physiol.*, **96**, 302.
SILVETTE, H. (1942), *Proc. Soc. Exp. Biol. Med.*, **51**, 199.
SOLEY, M. H., and SHOCK, N. W. (1938), *Amer. J. Med. Sci.*, **196**, 840.
STERN, K. G. (1940), *Ann. Rev. Biochem.*, **9**, 1.
Idem and DANCY, T. E. (1941), *Proc. Soc. Exp. Biol. Med.*, **48**, 619.
STONE, T. T., ARIEFF, A. J., and LUHAN, J. A. (1942), *Arch. Neur. Psychiat.*, **48**, 407.
STONE, W. E. (1938), *Biochem. J.*, **32**, 1908.
Idem (1940), *J. Biol. Chem.*, **135**, 43.
STRÖM-OLSEN, R., and KITE, E. DE C. (1942), *J. Ment. Sci.*, **88**, 407.
SUTHERLAND, E. W., COLOWICK, S. P., and CORI, C. F. (1941), *J. Biol. Chem.*, **140**, 309.
THOMPSON, V., and TICE, A. (1941), *J. Pharmacol.*, **73**, 455.
THORLEY, A. S. (1942), *J. Neurol. Psychiat.*, **5**, 14.
WACHTER, G. (1939), *Z. Kinderheilk.*, **60**, 623.
WAELSCH, H., SPERRY, W. M., and STOYANOFF, V. A. (1941), *J. Biol. Chem.*, **140**, 885.
WALLACE, G. B., and BRODIE, B. B. (1940), *J. Pharm. Exp. Therap.*, **70**, 418.
WATTERSON, D. J., and MACDONALD, R. (1939), *J. Ment. Sci.*, **85**, 1.
WEIL, A. (1941), *Endocrinology*, **28**, 150.
WOOD, H. G., WERKMAN, C. H., HEMINGWAY, A., and NIER, A. O. (1942), *J. Biol. Chem.*, **142**, 31.
WORTIS, J. (1941), *J. Neurophysiol.*, **4**, 243.
YOUNGSTROM, K. A. (1941), *ibid.*, **4**, 473.

https://doi.org/10.1192/bjp.90.378.64 Published online by Cambridge University Press