from the fact that any unfavorable condition of a tissue during growth is apt to lead to morphological change. It will be of interest to study, however, to what extent the chemical activities of the cell can be modified without any very marked morphological changes. To illustrate the above remarks I will give the analyses by the methods to be described later, of (1) a very young brain, (2) an adult brain, (3) a brain from a case of dementia praecox, representing a pathological condition in which there is no visible morphological change, but an unmistakable chemical variation.

The preponderance in the water-soluble-non-colloidal constituents in the growing brain over the adult brain is quite apparent. There is also to be noticed in the adult brain a relative increase of lipoids, especially cerebrin, cholesterol, and lipoid sulphur, all colloidal constituents which go to the formation of the medullated sheath, a morphological structure which plays an important rôle in the physiological differentiation of the cell.

The variation from the normal brain from dementia praecox is not nearly so striking, but nevertheless affects a group containing partially oxidized water-soluble sulphur compounds, the importance of which to the cell will be discussed more in detail in another paper.

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[From the Hull Physiological Laboratory, University of Chicago, and the Pathological Laboratory of the London County Asylums.]

## II. COLLECTION AND PRESERVATION OF MATERIAL.[1]

By W. Koch and S. A. Mann.

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*Changes Likely to Occur in Material Before Chemical Samples Can Be Collected.*—Whenever the circulation of the blood to a given tissue ceases or the tissue is removed from the body, the correlation of the different chemical reactions also ceases. Reactions of oxidation come to a stop and the hydrogen which was formerly depolarized by the oxygen with the formation of water now enters into reducing reactions. This is very well illustrated by Ehrlich's[2] observations that certain tissues which do not reduce alizarin blue during life, do so after death by the addition of two hydrogen atoms. There must also be substances normally present in the tissues which can be reduced in similar ways. It is not necessary to assume the presence of special substances in the tissues which accelerate reactions of reduction as distinguished from oxidation, for the very reaction which is so important in oxidation is the one which after death is responsible for reduction. Not only can such new reactions be in-

[1] For introduction see the preceding paper.

[2] Ehrlich, P., "Sauerstoff Bedurffniss des Organismus."

augurated but also such reactions as the hydrolysis of complex substances, which during life are counterbalanced by reactions of synthesis, can become exaggerated. Thus the liver if taken from the body immediately after death gives either none or only the faintest trace of sugar, while if allowed to lie only for a few minutes it will yield considerable quantities of reducing sugar. It is not always possible, especially when working with human material, to obtain the tissues so perfectly fresh as to avoid such changes as those of the glycogen mentioned above. Another type of change which it is practically impossible to avoid is the formation of an acid reaction in the tissues after death. This is partly due to the fact that after the stoppage of the circulation the carbon dioxide accumulates in the tissues and shifts the equilibrium in the phosphates to the acid phosphate.

$$Na_2HPO_4 + CO_2 + H_2O = NaH_2PO_4 + NaHCO_3.$$

It is also due to the formation of lactic acid by the hydrolysis of the sugar, which is derived from the glycogen as mentioned above, and has been shown by Hopkins and Fletcher[1] to take place with extreme rapidity. Fortunately, however, the fats and the proteins do not undergo quite such rapid changes. By keeping the material, if it is impossible to take it in hand immediately, at a low temperature, the speed of these reactions of hydrolysis can be considerably decreased. A certain amount of post-mortem change, especially in human material, will however always take place, and it becomes necessary to adopt the principle used in histology, namely that if material is taken in each case under comparable conditions the results though not of absolute value can be used for deductions involving comparisons. It is well for the chemist to realize, however, that his work, unlike that of the physiologist, has to do with dead material, and the sooner dead the better for the accuracy of his results. Hopkins' results on the lactic acid in muscle illustrate this in a striking manner. Only by previously cooling the alcohol and taking the frog's muscle from the body under conditions which avoid any stimulation is it possible to demonstrate the complete absence of lactic acid in living tissue. For this reason methods of analysis which render inactive, as soon as possible, all the substances that have to do with the acceleration of chemical reactions are to be preferred. This point will be again referred to later in connection with the preservation of material.

*Uniformity of Sample.*— When we take a tissue for analysis it is well to keep in mind the fact that we are not dealing with an anatomical unit. Indeed, only in rare instances, as for instance in the case of the heads of spermatozoa, or in certain lower plant forms (bacteria), can we obtain anatomically uniform material. Thus in animal tissues we have to consider at least four different anatomical elements: the blood,

[1] Hopkins, F. G., and Fletcher, W. M., *J. Physiol.*, **35**, 247 (1907).

the blood vessel elements, the connective tissue cells, and the parenchyma cells. It might indeed be possible to remove the blood by transfusion, but we would only substitute salt solution, which although free from organic substances would very seriously upset the accuracy of the estimation of inorganic salts. It is here again necessary to adopt the comparative method of histology and study the tissues as a whole. If we observe variations in the composition it will require histological or rather microchemical tests to decide whether the change is to be attributed to the parenchyma cells or to other groups of cells found in the tissue.

This, however, does not obviate the sources of error due to the varying amount of blood present in the tissue, under the different conditions that may obtain at the time of death. This affects a tissue like liver very considerably. Thus the liver from a case which has slowly bled to death may not be altogether comparable to the one from a case which died with the abdominal vessels filled with blood. In animal material it would be desirable in some way to estimate the amount of blood in the tissue and with a separate analysis of the blood itself as a basis, apply a correction. Occasionally it is desirable to separate in a rough way certain groups of anatomical elements; thus in the brain the gray matter, which contains the nerve cell, probably represents a more active center of metabolism than the white matter which consists mainly of conducting fibers. In investigating changes of the nervous metabolism under different pathological conditions it is obvious that the proportionate variation in the gray matter may be expected to be greater than when the whole brain is used. In this manner variations which might otherwise pass unnoticed or be attributed to the usual variations in the analysis, come more into prominence and can be detected. There are, however, very few tissues in which it is possible to apply this principle, and it is well to bear in mind that we will never be able to refer the changes observed in the tissues, as a whole, to certain of its anatomical elements until we have good microchemical tests at our command. It is well, however, to remember in this connection that microchemical tests must, by their very nature, be qualitative rather than quantitative and very much under the influence of personal error.

*Variations in Water Content of Tissue.*—As the results of chemical analyses of tissues from different cases have to be calculated in terms of the dry matter, in order to be comparable, it is necessary to make a moisture estimation at the same time that the material is taken for chemical analysis. This is especially important in those cases where the material is weighed in a moist condition.[1] The methods of making moisture estimations will be discussed in detail later.

*Sampling the Material.*—The greatest care must also be taken in collect-

[1] See also editorial *J. Ind. Eng. Chem.*, 1, 685.

ing the material to obtain a uniform sample. This presents much greater difficulties in investigations of tissues, than in the analysis of any other material that the chemist has to deal with, for the reason that it is impossible to grind fresh material to the degree of fineness that is possible, for instance, in the case of a sample of mineral to be analyzed. If the material is dried before the chemical samples are taken this difficulty can to a large extent be overcome, but, as will be discussed a little later, it is often necessary to collect material in a moist condition in order to obtain it sufficiently fresh. If we consider, however, that the larger the sample we take the greater will be its uniformity, the solution of the difficulty becomes possible. Great care, however, must be exercised to obtain a uniform mixture especially in tissues, which like muscle contain a lot of fat, more especially fat of a liquid nature. The best method of obtaining a uniform mixture is to grind the tissue two or three times through a good meat chopper. In case the material is to be dried it is better also to use the meat chopper first. Later, the dry material can be put through an ordinary coffee grinder and any degree of fineness obtained.

*Size of Sample.*—Guided by the necessity mentioned above of obtaining a uniform sample, it is well not to take samples which are too small. Some regard, however, must be had to the nature of the analysis. Thus if we wish to estimate phosphorus it is necessary that the weighing of magnesium pyrophosphate should give at least three figures, in other words, exceed 10 mg. as a minimum. It would indeed be better to try always to obtain 100 mg., but as this often involves the destruction of unusually large amounts of organic matter, the inaccuracies introduced by the handling of such large quantities overbalance the greater accuracy in the weighing of the final precipitate. In cases where the distribution of an element like phosphorus among the various groups is to be studied, it is necessary to take a large sample in order to obtain sufficiently accurate estimations in one group, while as regards another group it is necessary to take an aliquot part in order to avoid the difficulties of destroying large amounts of organic material. A little experience will, however, soon enable a chemist to select the proper mean.

*Weighing a Sample.*—A point that hardly seems worth mentioning, but which is often disregarded, concerns the accuracy of weighing the sample. Thus, if for instance a given sample of 10 grams is expected to yield 100 mg. of magnesium pyrophosphate, it is not necessary to weigh the sample to less than the second place of decimals or to a centigram. The time consumed in making a more accurate weighing is in no way compensated for by greater accuracy, but rather constitutes a source of error in a moist sample on account of the loss of water by evaporation.

An ordinary torsion balance which is capable of weighing 200 grams and is sensitive to 10 mgs. represents the best type for this work.

*Preservation of Material after Collecting Sample.*—Whenever feasible it is desirable to begin the chemical analysis as soon after the material is obtained as possible and with solvents which check the chemical reactions. Thus the methods used by Grindley[1] of beginning the extractions of the tissue with water, although they may do for muscle, especially at the low temperature which he employs, would hardly do for tissues which have a more rapid rate of autolysis. Very often, however, the amount of labor which must be concentrated into a short period of time in order to begin chemical analyses at once is so great that it is necessary to make use of a preservative. In the choice of a preservative it is necessary that the substance selected should in no way alter the solubility of the constituents, so as to interfere with the analytical methods to be employed later. This excludes such preservatives as formol, heavy metals or chromium salts so much used in histology. As there is no preservative which does not remove something from the tissues it is necessary to weigh the sample before adding the preserving fluid. Among preserving fluids alcohol is the best as it can be used in the later analyses, in fact, it represents the beginning of the chemical analysis. The following three then represent essentially the methods to be employed:

1. Preservation in alcohol after weighing moist sample.

2. Preservation of tissue by drying in a stream of hot air, or after freezing.[2]

3. Preservation of the tissue by the addition of a dehydrating substance such as anhydrous sodium sulphate or gypsum.

It seems desirable to discuss the advantages and disadvantages of the above methods for quantitative work.

1. *Advantages of Preservation in Alcohol.*—(*a*) In most estimations, especially in the determination of fat, the alcohol represents the first step in the course of the analysis and thus does not interfere with later manipulations.

(*b*) If the amount of alcohol to the quantity of tissue is so adjusted as to have the resulting liquid 85 per cent. alcohol, all ferments are precipitated and chemical reactions should cease.

(*c*) There is no danger of loss of volatile constituents.

(*d*) This method of preservation permits of the accurate estimation of the largest number of constituents as will be seen in the later chapters.

*Disadvantages of Preservation in Alcohol.*—(*a*) The great difficulty with which alcohol penetrates tissues, which necessitates a fine mincing

[1] Grindley, THIS JOURNAL, **26**, 1086 (1904).

[2] Shackell, *Am. J. Physiol.*, **24**, 325 (1909).

and frequent shaking after the alcohol has been added (I have sometimes found tissue in an advanced state of putrefaction when allowed to remain under a layer of 95 per cent. alcohol without frequent stirring).

(*b*) The difficulties of transporting alcohol-preserved samples, on account of the danger of leakage through glass stoppers. This for chemical work means loss of material and consequent inaccuracies in the analysis.

(*c*) The necessity of weighing out all the samples as needed later and the need of estimating the moisture. In case a lot of material comes in at once this may mean the concentration of considerable labor into a short period of time and consequently danger of inaccuracies due to carelessness.

(*d*) The danger that reactions of oxidation of the unsaturated fats may go on in alcohol as pointed out by Erlandsen.[1]

Such an oxidation would render the iodine figure inaccurate.

(*e*) Changes in solubility observed by me in work on brains.

| Time of analysis after collecting: | 3 hours. | 2 days. | 2 weeks. | 3 months. | 9 months. |
|---|---|---|---|---|---|
| Alcohol soluble..... | 42.7 | 38.4 | 36.9 | 35.9 | 33.6 |
| Alcohol insoluble..... | 57.3 | 61.7 | 63.1 | 64.1 | 66.4 |

(2) *Advantages of Preservation by Drying.*—(*a*) The possibility of obtaining the material in a very fine state of division and consequently a very uniform sample.

(*b*) The fact that it is not necessary to weigh out the samples at once as needed later.

(*c*) The elimination of the water estimation. If, however, the material is not kept in a vacuum desiccator over calcium chloride, there can be no certainty that it is absolutely dry.

(*d*) The decrease of the danger of oxidation of unsaturated fats as pointed out by Erlandsen. By keeping the material in a vacuum this can almost absolutely be avoided.

*Disadvantages of Preservation by Drying.*—(*a*) The danger of scattering laboratory dust over the material in the process of passing over the hot air.

This may completely vitiate nitrogen or sulphur analyses and interfere with a great many others.

(*b*) The danger of loss of volatile constituents, *e. g.*, $NH_3$.

(*c*) The danger of changes due to autolytic changes or bacterial action unless the material is dried very rapidly and kept absolutely dry.

(*d*) The difficulties of weighing this material accurately in a dry state on account of its tendency to take up water.

(*e*) The tendency of animal tissues to form sticky, gummy masses on drying, which are extremely difficult to extract with solvents.

[1] Erlandsen, A., *Z. physiol. Chem.*, **51**, 71 (1907).

*Example*—Lecithin in muscle.

| | Sample placed in alcohol direct. | | Sample dried in stream of warm air and then extracted. | |
|---|---|---|---|---|
| Lecithin..... | 4.1 | 4.4 | 3.4 | 3.2 |

3. *Advantages of Adding Sodium Sulphate or Gypsum.*—(*a*) In cases, as for instance milk, where the material contains such a large amount of water that it would unduly dilute the alcohol or would be difficult to dry in a stream of hot air, a thin paste is made by adding about $^1/_3$ the weight of anhydrous sodium sulphate and the drying then proceeds rapidly.

(*b*) In special investigations such as those of Schryver[1] on autolysis and Rosenheim[2] in his estimation of cholesterol in the brain.

*Disadvantages of Adding Sodium Sulphate or Gypsum.*—(*a*) The fact that it is only possible to make a limited number of analyses with material preserved in this way.

In planning an investigation a consideration of the above factors will decide the choice of the preservative. In the work on the brain it has been found best to use alcohol and the methods to be subsequently described are based on that method of preservation.

*Details of Method of Collecting and Preserving Material.*—30–100 grams of the tissue, ground fine and thoroughly sampled as discussed above, are placed in a large mouthed, preferably glass stoppered, bottle and sufficient absolute alcohol added to bring the concentration up to at least 85 per cent. The amount of alcohol to be added depends upon the moisture content of the tissue. Thus 100 grams of tissue containing 25 per cent. of moisture, would require from 200–300 cc. of alcohol. After adding the alcohol the material should be thoroughly shaken up at intervals and the next day should be warmed to 75° by placing in a basin of water and gradually raising the temperature. It is better not to begin the analysis less than two weeks or more than 3 months after collecting the sample. It is well to make a note as to the time which elapses between the collection and analysis of the material, as some changes take place. (See alcohol preservation, disadvantage, *e*.)

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[From the Hull Physiological Laboratory, University of Chicago.]

## III. ESTIMATION OF THE PROXIMATE CONSTITUENTS.[3]

By W. Koch and Emma P. Carr.

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The six main groups of proximate constituents into which we have provisionally divided the constituents of a tissue are (1) Lipoids, (2) Ex-

[1] Schryver, S. B., *Biochem. J.*, **1**, 131 (1906).

[2] Rosenheim, O., *J. Physiol.*, **34**, 105 (1906).

[1] For introduction, see preceding papers.