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THE CHEMISTRY OF THE BRAIN IN THE MENTAL DEFECTIVE *

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INTRODUCTION

THE work done hitherto on the chemistry of the brain in its relation to intelligence is comparatively small. Most of it has been undertaken to demonstrate chemical differentiation during growth, and for this purpose the material used has been either animal brains both before and after birth, or human brains at various stages of maturity. We have made chemical analyses on 71 brains, 62 of them being from mental defectives and the remaining nine from normal controls. There are several points of view from which the results may be of interest.

1. The studies of many workers have shown that the chemical constituents of the brain undergo systematic changes during the development of the individual. Some results are available for the human brain, and it will be of interest to see whether the mental defective shows changes in the chemistry of his brain which would relate the varying mental ages to the chronological ages of normal children. In other words, it will be of interest

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to see whether the defective can be considered to be infantile in his brain-chemistry.

2. Some of the main histological features of the cerebral cortex are very difficult to measure or assess objectively, particularly the richness in cells and the degree of myelinization of the fibres, as it is almost impossible to stabilize the intensity of the staining. Some problems of this type, however, may be answered with greater accuracy and directness by chemical analysis.

PREVIOUS WORK

The sole observations with which we are acquainted on the chemical composition of the brain in the mental defective are those of Tilney and Rosett.¹⁶ In their work, they used 68 human brains which had been preserved in formalin for different lengths of time. Before analysis they were divided into brainstem, right and left cerebral hemispheres, and right and left halves of the cerebellum, but no division was made between white and grey matter. Estimations made to determine the water, lipoid, and non-lipoid values showed that two brains from low-grade defectives were not significantly different from the normal adult brains.

The changes in the lipoids of the human brain during development have been investigated by Schiff and Stransky,¹⁵ Smith and Mair,¹⁴ and Koch and Mann.¹¹ Their results show that during growth there is a decrease in water content and an increase in lipoids. Similar results have been obtained by several workers using the brains of animals, notably by Koch and Koch,¹⁰ Donaldson,⁴ and Backlin.² Further Koch and Koch, and Donaldson, working on brains, state that the water decrease is in progress before medullation begins, and according to Donaldson the process of medullation is not a controlling factor in reducing the percentage of water in the nervous system. Gorodisskay⁷ has done considerable work on the brain lipoids, making the analyses on specific areas, but her work is not connected with development or mental deficiency. Koch and Mann¹² have also shown that no important changes occur in the brain lipoids up to 30 hours after death.

TREATMENT OF THE BRAINS BEFORE ANALYSIS

In collecting a series of brains of mental defectives it has not been possible to examine them in a fresh state, chiefly because of the length of time taken to collect the series. It was also necessary to harden the brains in order to be able to separate the grey from the white matter, since fresh brain tissue is too friable to enable this to be done successfully. All the brains had been kept in 10 per cent. formol saline, which was changed repeatedly. The fact that the brains have been preserved in formalin is certainly disadvantageous, but need not invalidate everything. What we have done is to record the time in formalin for each brain, and then, by using the statistical methods of partial regression, we have analysed out separately the effects of mental age and of time in formalin on each particular chemical constituent.

Thus, in the case of the cerebrosides, we wish to study the relationship between cerebroside content and mental age, the time in formalin being a possible complication which we wish to remove. Fitting the equations for the partial regressions of cerebroside on mental age, and of time in formalin, we obtain the effect of each, independently of what the other is doing. Since the equation gives the effect (if any) of time in formalin on cerebroside, we are able to correct the cerebroside figures to what they would have been had all the brains been in formalin for the same length of time. In this way, although we cannot say what the results would have been had we dealt with fresh brain, yet our results are at least corrected to a standard time in formalin.

It will be seen that while our results are not as satisfactory as analyses of fresh material would be, yet by using these methods of statistical analysis results may be obtained which are valid, subject to the restriction that we are dealing with results corrected to a *standard* time in formalin and not corrected to the fresh state.

On most of the substances formalin appeared to have little or no effect. In these cases our results are probably almost as good as if the analyses had been done on fresh material. The phosphatides, however, are considerably affected. These results will be discussed more fully later in connexion with each particular constituent.

These same 71 brains are also being examined from many other points of view. Since a general description of them is being published elsewhere ¹ it is not necessary to repeat here all the data such as sex, age at death, etc.

We cut out a block, about $1\frac{1}{2} \times 1$ inch in size, from the medial surface, anterior to the corpus callosum, of the right hemisphere of each brain. The frontal lobe was chosen as it is commonly considered to be the most interesting region in defectives. A block of this size provides about 8 gm. of grey matter. This is obtained by cutting the block into slices and then carefully trimming off the grey matter. For this purpose we have found a von Graefe cataract knife to be convenient. Some preliminary experiments, done by shaving the cortex into layers, showed that the chemical composition of the cortex remained practically constant as one descended from the surface towards the white matter and that it tended to increase fairly sharply just as one approached the white matter. It is therefore better in dissecting off the grey matter to keep about $\frac{1}{2}$ mm. above the white matter. Losing a little grey matter makes practically no difference, but the inclusion of a small amount of white matter would upset the results considerably.

CHEMICAL METHODS

In most of the analyses described below, the values obtained in each case probably represent a group of compounds rather than a single one. As the group is precisely determined by its method of estimation, this distinction in no way alters the validity of the results, but it is one which must be

remembered. Should the values for any group be significantly correlated with mental age, then that group could be more exhaustively examined. Should no such correlation be found, then its absence refers to the group as a whole.

After the grey matter required had been removed from the brain, it was stripped of any adhering membranes and was washed overnight in running water to remove formalin. It was then ready for drying and extraction.

Water Content.—This analysis was performed in duplicate, and for each estimation 0.3 to 0.4 gm. of the material was used. The brain was superficially dried with filter paper, rapidly spread on to a tared filter paper strip, and weighed. This strip was contained in a weighing bottle and was counterpoised with a similar bottle and filter paper strip. After weighing, both bottles were placed in a vacuum desiccator and the brain was dried to constant weight. Usually two days were sufficient.

From the results of the 71 duplicate estimations the standard deviation and coefficient of variation were calculated. They are : $s = 0.532$; C. of V. = 0.64 per cent. (In calculating the standard errors we have used 'degrees of freedom' according to Fisher's method⁵ ; older methods give a figure which seriously underestimates the true error.) These are the errors of the method. Since our final results, however, are the means of two estimations, the error actually affecting our figures will be $s/\sqrt{2} = 0.383$.

Drying.—The washed brain was cut into small pieces, and after being superficially dried on filter paper was placed in a vacuum desiccator for one day. The pieces were then finely ground in a mortar and returned to the desiccator until constant in weight. By this method, 8 gm. of material were completely dried in 10 days.

Extraction.—Chloroform was used for the extraction in preference to any of the other solvents which have been more generally employed. It seems more desirable to carry out the extraction with one solvent in which all the known lipoids are soluble than to use a series of solvents such as acetone, alcohol, ether and petroleum ether, each of which dissolves only a fraction of the total lipoids. The objection to using a number of solvents is that these lipid substances considerably affect the solubility of each other, thus preventing a reliable fractionation. For example, it is well known that although kephalin is ordinarily insoluble in alcohol, it will dissolve in the presence of lecithin. It has also been shown by Maclean¹³ that although the phosphatides are insoluble in acetone, lecithin is soluble to a small extent in acetone which contains fatty substances in solution. In view of these complications it was decided to make the estimations without isolation of the groups to be studied. In any case there is not much to be gained by such isolation since the quantities obtained would be too small to weigh and would have to be estimated by indirect methods.

All the estimations were made on 0.8 to 1.0 gm. of dry brain. This was weighed into a 100-ml. CO₂ flask and was extracted with 50 ml. of chloroform.

The flask, fitted with a reflux condenser, was heated in a water bath kept at about 70° C. After three and a half hours the chloroform was filtered through a fat-free filter paper into another 100-ml. flask of known weight. The brain residue was returned to the extraction flask and heated with a fresh quantity of chloroform for five hours. In all, four lots of chloroform were used and the extraction time was 18½ hours. Further extraction was found to be superfluous. After extraction of the brain, the filter papers used were extracted with chloroform for one hour and this extract was added to the main one. During each extraction the chloroform of one was allowed to evaporate down to about 10 ml. by being kept in a water bath at 55°–60° C. After the final extraction the last 10 ml. were allowed to evaporate at room temperature. The extract was then dried in a vacuum desiccator to constant weight. This method possibly requires more attention than the various Soxhlet methods, but it gave very reliable results.

From four estimations on one sample and five duplicate estimations on different samples the standard deviation and coefficient of variation were calculated. They are : $s = 0.209$; C. of V. = 0.80 per cent.

Analysis of the Extract.—When its weight had been determined the extract was redissolved in chloroform and made up to 50 ml. From this solution aliquots were used for the determination, in duplicate, of cholesterol, cerebroside and phosphatides.

Cholesterol.—It was at first intended that the digitonin method should be used as it is more accurate than colour methods and in addition it estimates the cholesterol free from its esters. In practice, however, this method is very slow and tedious, and its accuracy is by no means in proportion to the time required. By making duplicate estimations with the colour method a sufficient accuracy could be obtained in a fraction of the time.

Grigaut's ⁸ method, based on the Liebermann reaction, was adapted for use as follows : 5 ml. of the extract were diluted to 50 ml. ; to 5 ml. of the diluted solution were added 2 ml. of acetic anhydride followed by 0.1 ml. of concentrated sulphuric acid ; the constituents were well mixed and the colour allowed to develop in the dark for half an hour. The standard for comparison was 5 ml. of a chloroform solution containing 0.00012 gm. of cholesterol per ml., and this was treated in the same way as the sample. After half an hour the intensities were compared in a Bausch and Lomb colorimeter, using a red filter, Wratten 'A,' to eliminate a yellow tint in the brain extract.

From the 71 duplicate estimations, $s = 0.189$; C. of V. = 2.93 per cent. Since each of our results is the mean of two estimations, the error actually affecting our results is $s/\sqrt{2} = 0.134$.

Cerebroside.—In the estimation of cerebroside it is usual, when there is sufficient material, to extract this fraction and weigh directly. With less material there are two methods available. The first ⁹ consists in hydrolysing with acid and estimating the liberated galactose. In the second method the

cerebrosides are calculated by difference and everything else present in the fraction must first be determined. In this method the total lipoids are estimated on an aliquot part by Bang's³ chromic acid oxidation method. From the value obtained by this method the previously estimated lipoids are subtracted and the difference is designated as cerebrosides. Clearly this method can only be used when everything in the extract can be estimated. This is not the case in a chloroform extract which contains a number of undetermined fats and waxes. A method involving hydrolysis and estimation of galactose had therefore to be used and a modification of Koch's⁹ method was chosen.

Ten ml. of the extract were pipetted into a pyrex boiling-tube of dimensions 15 × 85 mm. and allowed to evaporate to dryness. Eight ml. of 15 per cent. sulphuric acid were added to the residue, which was hydrolysed by heating gently with a micro burner for one hour. A condenser of the hanging glass type was used. To facilitate filtration, 3 ml. of saturated sodium sulphate solution were added and after filtration the solution was neutralized with caustic soda and made up to 50 ml. Five ml. of this were used for the sugar estimation, which was done colorimetrically by the Folin and Wu method. The standard for comparison was 5 ml. of a glucose solution prepared by diluting 2 ml. of a 1 per cent. solution to 1 litre. The values quoted in the literature vary enormously. The results of the present method (mean = 2.42 per cent.) agree quite well with the values found by Smith and Mair¹⁴ by direct weighing (mean = 1.94 per cent.).

From the results of 65 duplicate estimations, $s = 0.0666$; C. of V. = 2.70 per cent. Since our final results are the means of two estimations, the error actually affecting the final results is $s/\sqrt{2} = 0.0471$.

Phosphatides.—These were determined by estimation of the contained phosphorus. It was assumed for the purpose of calculation that all the phosphorus was present as lecithin. This does not, of course, represent the true circumstances, but that is not important for the present work. Actually the results could have been recorded as phosphorus values.

Three ml. of the extract solution were pipetted into a pyrex boiling-tube of dimensions 10 × 200 mm. After the chloroform had evaporated the residue was incinerated over a micro burner with 1 ml. of concentrated sulphuric acid. When the charring was complete, 1 or 2 drops of nitric acid were added to complete decomposition. When quite colourless the solution was cooled, diluted with water and neutralized with caustic soda using phenolphthalein as indicator. The neutral solution was transferred to a 50-ml. flask and the blue colour developed by the addition of ammonium molybdate and amino-naphthol-sulphonic acid according to the method of Fiske and Subarow.⁶ The standard for comparison was a known volume, 2 to 3.5 ml., of a solution of potassium dihydrogen phosphate containing 0.08 mg. phosphorus per ml. The colour developed in about 15 minutes and remained constant for several hours.

From the results of 45 duplicate estimations, $s = 0.198$; C. of V. = 1.99 per cent. Since our final results are the means of two estimations, the error actually affecting our results will be $s/\sqrt{2} = 0.140$.

Nucleoproteins.—When the lipoids have been extracted, the phosphorus remaining in the residue may be considered to represent the nucleoproteins and inorganic phosphates. It was intended to estimate the latter by boiling a portion of the residue in water and estimating the extracted phosphorus. In another portion the total phosphorus was to be estimated by incineration as described above for phosphatides. By difference an estimation of the nucleoproteins would be obtained.

For the total phosphorus estimation about 0.05 to 0.06 gm. of the residue was used. This was weighed into a pyrex boiling-tube and incinerated with 1.5 ml. of strong sulphuric acid together with a crystal of potassium sulphate. When colourless, the solution was made up to 50 ml., of which 20 ml. were used for the colour estimation. In attempting to estimate the inorganic phosphorus it was noticed that the water extract became faintly turbid on adding the acid ammonium molybdate solution, and that after adding the amino-naphthol-sulphuric acid no colour developed until about a day had passed and even then it was too faint to measure. Several of the brain residues were extracted, but none contained any appreciable inorganic phosphorus. We have therefore concluded that the inorganic phosphates must have been dissolved out of the brains during their preservation in formol saline, and the total phosphorus itself is taken to represent the nucleoproteins.

The experimental error of estimating protein phosphorus has been measured by performing six incinerations on one sample of brain, and then making two colorimeter estimations on each incineration. By testing in this manner it is possible to analyse out separately the error between incinerations and the error between colorimeter estimations. After calculating the errors, we find the variance between incinerations to be 85.2×10^{-6} , and variance between colorimeter estimations to be 68.4×10^{-6} . We used one incineration and one colorimeter estimation on each of our brains, so the variance of the final result is $85.2 + 68.4 = 153.6 \times 10^{-6}$, giving standard deviation = 0.0124, and coefficient of variation = 2.82 per cent.

TABLES OF RESULTS

The results of the analyses are given in Table I.

Contrary to many authors, we are of the opinion that the results for the constituents are best all given in the form of 'gm. of substance per 100 gm. of fresh (undried) tissue.' Our reason for this is that there is no point in working out ratios which are uninterpretable. To be of any use, apart from mere recording purposes, the data must be in a form suitable for interpretation, usually from the physiological point of view. In physiology we are always dealing with living tissues where the water is absolutely as essential and important as, say, the proteins. Consequently, to express one's results as so

much after the water has been eliminated is to give them in a form which corresponds to nothing in nature.

For recording purposes it hardly matters which method is chosen since one may soon convert the figures from any one type to any other.

In Table I are given :—

- (A) Patient's reference number. These agree with the other data published elsewhere about the same brains.
- (B) Mental age in years.
- (C) Water content in gm. per 100 gm. of undried brain.
- (D) Total chloroform extract (same units).
- (E) Total (dry) residue after extraction (same units).
- (F) Cerebrosides (same units).
- (G) Phosphatides (same units).
- (H) Cholesterol (same units).
- (J) Protein phosphorus (same units).
- (K) Time in formalin. Here is given $\log_{10}$ (time in months), for reasons furnished later.

ERRORS

There are two separate types of error to be considered : (1) Errors in the chemical analyses, and (2) errors in handling the group results.

1. In the analyses we have to consider first whether the analysis gives the result for all of the substance and for nothing else. As pointed out above, this may be judged from the descriptions of method and need not be considered further.

The second point to discuss is whether the analyses are sufficiently accurate. The accuracy of all the methods has been measured and the errors have been given above in the form of the standard deviation and the coefficient of variation. We have to decide whether the accuracy is sufficient. We have approached the subject on the following lines.

The effect of the errors of estimation is essentially to waste information, e.g. if we examine 71 cases using a method with a certain experimental error, then the final effect, for correlation purposes, is exactly the same as if we had measured a smaller number of cases with infinite accuracy. Now our particular purpose is to establish whether or not there is any significant correlation between mental age and the percentage of substance in question. Should a significant correlation be found between the two variables, then the question of experimental error need not trouble us further since the effect of such errors is to obliterate correlations and it would mean that the correlation had appeared in spite of the errors. On the other hand, should the correlation prove insignificant, we must then inquire how much correlation might be present, for one can never, of course, be sure that there is absolutely no correlation present. But by using a suitable number of cases one can ensure

TABLE I

No. A	Mental age B	Water content C	CHCl ₃ extract D	Total residue E	Cere- brosides F	Phos- phatides G	Chole- sterol H	Protein F J	Time in formalin K
1	1	84.4	3.69	12.0	0.348	1.05	1.01	0.0642	1.93
2	1	82.7	4.18	13.1	0.396	1.24	1.12	0.0734	1.86
3	1	82.4	3.62	14.0	0.435	—	0.86	0.0755	1.56
4	3	84.5	3.87	11.7	0.335	—	0.99	0.0671	1.69
5	4	83.6	3.81	12.6	0.396	0.99	0.87	0.0695	1.86
6	4	84.2	3.99	11.8	0.371	1.18	1.10	0.0626	1.91
7	5	82.7	4.40	12.9	0.386	—	1.12	0.0726	1.76
8	5	81.9	4.39	13.7	0.439	—	1.12	0.0778	1.80
9	7	85.2	4.08	10.7	—	—	0.92	0.0572	2.01
10	8	83.8	3.89	12.1	0.390	—	0.98	0.0691	1.71
11	9	84.3	3.83	11.9	—	—	0.89	0.0644	1.69
12	1	85.6	3.61	10.8	—	—	0.84	0.0578	2.00
13	5	83.0	3.92	13.1	0.357	0.48	1.01	0.0676	1.92
14	7	83.7	4.10	12.2	0.383	0.89	1.17	0.0697	2.01
15	3	81.7	4.22	14.1	0.376	1.30	1.23	0.0711	1.64
16	3	82.5	4.25	13.3	0.442	1.20	1.21	0.0778	1.59
17	12	83.3	3.85	12.8	0.336	1.11	1.00	0.0726	1.38
18	2	85.7	3.76	10.6	—	—	0.72	0.0656	1.34
19	5	86.2	3.56	10.3	0.322	—	0.91	0.0617	1.45
20	5	84.2	4.18	11.6	0.394	1.42	1.00	0.0684	1.52
21	8	87.0	3.64	9.3	—	—	0.74	0.0579	1.28
22	5	85.8	3.86	10.4	0.344	—	0.90	0.0594	1.34
23	3	84.2	4.06	11.7	0.382	—	1.02	0.0728	1.38
24	3	83.8	4.06	12.2	0.384	1.36	1.04	0.0695	1.51
25	5	84.4	3.95	11.7	0.372	—	0.99	0.0681	1.40
26	5	84.9	4.10	11.0	—	—	0.90	0.0674	1.30
27	6	85.2	3.90	10.9	0.360	—	1.00	0.0665	1.32
28	8	85.3	3.91	10.8	0.415	—	0.98	0.0644	1.38
29	4	83.6	4.14	12.2	0.435	1.23	1.10	0.0763	1.54
30	2	82.7	4.43	12.9	0.456	1.56	1.11	0.0779	1.53
31	2	84.7	3.76	11.5	0.392	—	0.98	0.0688	1.34
32	3	84.3	3.84	11.7	0.402	—	0.91	0.0682	1.40
33	5	85.0	3.86	11.2	0.335	—	1.04	0.0671	1.36
34	9	84.1	3.89	12.0	0.409	—	0.96	0.0727	1.34
35	3	84.8	3.73	11.5	0.312	—	0.80	0.0706	1.30
36	3	84.8	4.06	11.2	0.328	—	0.97	0.0665	1.30
37	4	85.6	3.70	10.7	0.380	—	0.98	0.0632	1.38
38	0	86.2	3.58	10.2	0.282	—	0.91	0.0644	1.38
39	1	83.9	4.05	12.0	0.402	1.46	1.02	0.0694	1.51
40	2	84.1	3.98	11.9	0.435	1.36	1.03	0.0803	1.51
41	4	85.6	4.17	10.4	0.429	1.70	1.06	0.0618	1.46
42	2	84.4	4.17	11.5	0.390	1.55	1.15	0.0676	1.43
43	2	82.5	4.58	12.9	0.464	1.60	1.16	0.0765	1.43
44	6	83.2	4.39	12.4	0.452	1.49	1.21	0.0767	1.34
45	7	83.0	4.47	12.6	0.397	1.42	1.16	0.0713	1.32
46	3	83.3	4.39	12.3	0.358	1.63	1.18	0.0766	1.32
47	5	84.7	3.96	11.3	0.440	1.61	0.84	0.0689	1.28
48	0	84.7	3.76	11.6	—	1.68	0.88	0.0716	1.26
49	3	83.9	3.84	12.3	0.305	1.43	0.99	0.0735	1.26
50	6	84.2	4.14	11.7	0.385	1.58	1.00	0.0778	1.34
51	3	84.1	4.23	11.7	0.400	1.67	1.07	0.0717	1.23
52	8	84.2	4.04	11.8	0.386	1.55	0.96	0.0711	1.30
53	1	82.6	4.60	12.8	0.408	1.76	1.19	0.0765	1.23
54	4	85.6	3.73	10.7	0.347	—	0.93	0.0645	1.15
55	6	83.7	4.35	12.0	0.331	2.13	1.06	0.0810	1.11
56	1	83.8	4.27	11.9	0.377	1.71	1.11	0.0772	1.23
57	3	84.2	4.26	11.6	0.430	1.61	1.05	1.0707	1.26
58	0	85.4	3.91	10.6	0.311	1.69	0.98	0.0643	1.08
59	1	84.2	4.27	11.6	0.402	1.87	1.14	0.0767	0.95
60	9	82.2	5.10	12.8	0.506	2.47	1.18	0.0759	0.90
61	7	82.6	4.86	12.5	0.372	1.88	0.87	0.0768	1.04
62	0	84.8	4.00	11.2	0.377	2.04	1.00	0.0767	0.85
63	14	82.9	4.54	12.5	0.478	1.81	1.25	0.0794	1.04
64	14	84.7	4.80	10.5	0.367	2.61	1.07	0.0563	0.70
65	14	82.1	4.74	13.1	0.575	1.82	1.33	0.0808	1.00
66	14	82.4	4.61	13.0	0.430	1.91	1.16	0.0817	0.85
67	14	82.6	5.04	12.3	—	—	1.21	0.0755	1.11
68	14	84.3	4.54	11.2	0.359	2.75	1.06	0.0650	0.60
69	14	83.0	4.80	12.2	0.518	2.10	1.29	0.0779	0.78
70	14	84.1	4.47	11.4	0.378	—	1.03	0.0785	0.60
71	14	85.6	4.71	9.7	—	2.40	1.06	0.0515	0.78

that if a correlation has not been demonstrated, then any correlation present must be less than a certain amount. For reasons discussed elsewhere,¹ we

have decided that ' $r^2 = 0.1$ ' is a suitable limit for this type of work. We have shown that this limit can be guaranteed provided that the fraction

$$\frac{\text{experimental variance}}{\text{total variance between patients}}$$

does not exceed 0.135. This fraction has not been exceeded by any of our methods. We may therefore conclude that the accuracy of the methods is sufficient.

2. A second source of error, much more important than the above, is the error of random sampling. Our 71 cases are only a sample from the total population of defectives, and samples may have, by chance, all sorts of small peculiarities not present generally. Proper tests of significance, however, will tell how much is properly ascribable to chance, and only if the features exceed this amount can the findings be considered significant. Since, in our case, we wish to test the partial correlations with time in formalin eliminated, we have used Fisher's ⁵ tables, which are very convenient.

In eliminating the effect of time in formalin, we found that the (partial) regression of phosphatide on time did not appear to be linear. On testing it against $\log_{10}$ (time in formalin), however, the regression became linear. Since it is much easier to calculate and handle linear regressions, we have used the logarithm of the time in formalin throughout. We have examined all the other regressions and have satisfied ourselves that they are reasonably linear.

There is one other source of error which needs mention. Our particular series of cases is biased for epilepsy, in that this condition occurs mostly at the lower end of the mental age scale. In order to make sure that the epileptics were not peculiar in their chemical 'make-up,' we have calculated the residuals of mental age and substance after eliminating $\log$ (time) and have then examined the figures for the epileptics to see whether they were in any way different. It is scarcely necessary to give all the figures here, and we may state at once that the epileptics appeared to be just the same (chemically) as the other cases, having regard to their mental age and to time in formalin.

DISCUSSION OF THE RESULTS

We may now proceed to our findings in each substance for correlation between amount of substance and mental age.

Water Content.—The partial correlation between water content and mental age, in our series, with time in formalin standardized out, is -0.358 . This value is well beyond the 1 per cent. point of random sampling and is therefore undoubtedly significant. We can, therefore, be certain that there is some real connexion between the two columns of figures. As far as we can see, there is only one way in which this finding could be fallacious, and that is that there might be some differential effect in formalin. A general shrinkage could not produce a correlation; there would have to be a differential

effect, so that those brains of higher mental age responded to formalin differently from those of lower mental age. By examining a three-dimensional graph of the cases and the interrelations between the three variables, we have satisfied ourselves that no differential response occurs in the period covered by our cases. Any differential response, if it exists, must occur early in the fixation process. The formalin effect is small in any case, the partial correlation between log time and water content, mental age eliminated, being insignificant.

Although the validity of the correlation could only be established

FIG. 1.—Dot diagram showing the water content of 71 brains of varying mental ages. The nine normal cases have been given a mental age of 14. Water content has been corrected for time in formalin.

definitely by reexamining a new series of fresh brains, yet, since one does not usually expect these peculiar effects, we think that there is probably a correlation between mental age and water content of the grey matter.

Fig. 1 shows the 71 cases with water content plotted against mental age. The values plotted for water content are not those of column C in Table I, but have been corrected for the formalin effect so as to display the relation more clearly. Each dot represents one brain.

The partial correlation is negative and this means that as mental age increases, the water content, on the average, falls off. This is certainly interesting, since the same falling off of water content is known to occur quite generally in the development of the brain.^{2, 4, 10, 11, 14, 15} The general significance of this change is not known. The fall in water content in normal

development is certainly *not* due, as is commonly supposed, to increasing myelinization, since the water content commences to fall before myelinization commences.^{4, 10} It seems to be associated with some much more profound change, possibly in the nature of the proteins of the brain. To study the point further would require further research, but the change is certainly interesting.

Before we proceed to discuss the other results, we must first take account of a complicating factor. As the water content shows a significant correlation with mental age, and as any alteration in one constituent is bound to be accompanied by an inverse alteration in the others, so the other figures must first be freed from the water effect. This we have done by recalculating all the quantities as so much per cent. of dry weight. This eliminates any connexion with the water content and enables us to test for correlations quite apart from the disturbing water variation.

All the subsequent correlations are calculated using per cent. of dry weight.

Extract.—The extract content is undoubtedly affected by time in formalin (the partial correlation with mental age eliminated is -0.543). The correlation between mental age and extract is insignificant after eliminating the time in formalin effect. This agrees generally with the results of Tilney and Rosett,¹⁶ who analysed two defectives' brains but found no change in the lipid content. This absence of change is of interest since the figures for extract probably represent 'myelin' fairly closely. In our series, therefore, there is no evidence of any falling off in myelinization in the lower mental ages. As we mentioned previously, the value of this estimation lies in the fact that it is hardly possible to get at the 'degree of myelinization' in any other way with reasonable accuracy. The fact that we have found no significant correlation between mental age and amount of extract suggests, therefore, that the mental defective does not differ appreciably from the normal in the degree of myelinization of the grey matter.

Cholesterol.—The cholesterol content of the grey matter appears to have no correlation with mental age, after eliminating time in formalin (the correlation is $+0.078$, which is quite insignificant). This, of course, is to be expected with no change in myelin, since cholesterol is one of the main constituents of myelin. Time in formalin appears to have no effect on the cholesterol content (the correlation with mental age eliminated is -0.192 , which is insignificant).

Phosphatides.—Phosphatides are markedly affected by time in formalin (correlation, eliminating mental age, is -0.852). On eliminating the time in formalin effect, no correlation is found between mental age and phosphatide content (actually the correlation is -0.077). The phosphatide content, therefore, does not appear to be appreciably changed in the mental defective.

Cerebrosides.—Cerebrosides do not appear to be at all affected by time in formalin (correlation is -0.051 , which is quite insignificant). They show no significant correlation with mental age (actually the correlation is $+0.162$,

which is insignificant). This is of interest since in normal development it is the cerebrosides which show the most marked change between birth and maturity. Most of the work has been done on animals, but Smith and Mair¹⁴ find the cerebrosides to increase about 300 per cent. from birth to maturity in man. The change in cerebrosides in normal development is so marked that any reasonable correspondence in our series could hardly be missed. The negative finding, therefore, is fairly conclusive in showing that the mental defective's brain does not correspond chemically with the brain of the normal child.

Phosphatide-cholesterol Ratio.—Since, in many respects, phosphatides and cholesterol have antagonistic physicochemical properties, we have examined the phosphatide/cholesterol ratio in our brains to see if there is any systematic alteration. On testing the correlation of this ratio with mental age (time in formalin eliminated), the correlation is insignificant, indicating no particular change with mental age.

Since this ratio is of particular interest in the epileptics, we have recalculated the P/C ratio, eliminating both mental age and time in formalin, and have examined the residuals to see if the epileptics showed any difference from the non-epileptics. No significant difference was found.

Protein Phosphorus.—On testing the correlation of protein phosphorus with mental age, time in formalin eliminated, we find the correlation to be — 0.386, which is definitely significant.

This is certainly interesting since protein phosphorus represents largely nucleoprotein and the figures given in Table I will be approximately proportional to the amount of nucleoprotein present in the cortex. It appears, therefore, that there is some alteration in the content of nucleoprotein as mental age alters. It will be observed that the correlation is negative, indicating that the higher the mental age the less the amount of nucleoprotein. This is apt to appear surprising at first, since one would scarcely expect mental age to increase as something else falls off, but it must be remembered that we are dealing with 'gm. of protein phosphorus per 100 gm. of dry brain,' i.e. with a *relative* proportion, and what is true relatively is not necessarily true for absolute quantities. If we convert the correlation to give actual values, we find that in going from 0 to 14 years of mental age, the protein phosphorus per 100 gm. drops by 9.2 per cent. while the weight of the cerebrum increases by 9.3 per cent., indicating that the *total* quantity of nucleoprotein remains about constant. It appears, therefore, that the relative amount of nucleoprotein falls off while the total quantity remains constant.

It seems more likely, therefore, that what is really happening is that some other constituent of the brain is increasing with mental age and thus producing a relative diminution in the percentage of nucleoprotein. On considering what other constituents there are which could vary sufficiently to make an appreciable difference to the nucleoprotein, we may note that the lipoids may

be ruled out at once both collectively and individually since they have been tested directly and have shown no significant change. The only other constituent present in sufficient quantity is the 'non-nuclear' proteins. Consequently, we have considered the problem of estimating the non-nuclear proteins to see if we could test them directly for any correlation with mental age. Unfortunately there is no method for estimating them directly. They might possibly be estimated by considering the total protein and subtracting the amount of nucleoprotein, but the values obtained would depend almost entirely on the value chosen for the constant used to turn phosphorus values to nucleoprotein values.

In this connexion we may remember the point mentioned previously, viz. that there is no sense in calculating ratios which are subsequently uninterpretable. But there is one ratio here which is interpretable, and that is the ratio of nucleoprotein to non-nuclear protein. A nerve cell may be considered to be composed of two parts—a nucleus, and a cell-body with fibres (dendrons, axon). The amount of nucleus will correspond fairly closely with the amount of nucleoprotein, while the amount of cytoplasm will correspond approximately with the amount of non-nuclear protein. If the relative amounts of these two parts vary, then the ratio of nucleoprotein to non-nuclear protein will vary. Equally, if the ratio varies, then we may legitimately consider whether the variation could be due to an alteration in the relative proportions of nucleus and the rest of the cell. Further, if one cell had more prolongations than another, while the nuclei remained the same, then the ratio would alter in such a way that we might interpret it as a *relative* falling off in the amount of nucleus.

As we had already found a relative falling off in the amount of nucleoprotein as mental age increased, and as an interesting interpretation could be offered, we decided to test the point directly by calculating the ratio of nucleoprotein to non-nuclear protein to see whether the ratio showed any significant change with mental age.

As explained above, it was not possible to find explicitly the amount of non-nuclear protein, but for testing the ratio from our point of view this does not matter. We have :

$$\text{Total protein} = \text{nucleoprotein} + \text{non-nuclear protein}$$

and since the fraction $\frac{\text{nucleoprotein}}{\text{non-nuclear protein}}$ is a function of the fraction $\frac{\text{nucleoprotein}}{\text{total protein}}$,* we may use the latter just as well as the former. Both these latter quantities are known directly from the analyses.

*

$$\frac{x}{x+y} \text{ is a function of } \frac{x}{y} \text{ since } \frac{x}{x+y} = \frac{\frac{x}{y}}{\frac{x}{y} + 1}$$

Calculating the fraction for each patient and correlating them with mental age, we find the correlation, with time in formalin eliminated, to be -0.387 , which is definitely significant. It is to be noted that this correlation is practically the same as the previous one (-0.386) when we correlated amount of nucleoprotein with mental age. This is important, since in the fraction used above we have, in effect, divided each nucleoprotein figure by another (varying) figure; if the non-nuclear protein had really no connexion with the subject, then the introduction of irrelevant figures would have 'diluted down' the correlation and the final correlation would have dropped considerably. We see, therefore, that there is reason to believe that the non-nuclear protein is playing some definite part in the production of the correlation. Further,

FIG. 2.—Shows 'gm. of non-nuclear protein per gm. of nucleoprotein' (corrected for time in formalin) plotted against mental age. Each dot represents one patient.

this suggests that the ratio is a more natural method of examining the nucleoprotein-mental age relationship than considering the nucleoprotein by itself.

For purposes of interpretation we may note that the above fraction is equivalent (inversely) to 'gm. of non-nuclear protein per gm. of nucleoprotein.' This quantity is plotted against mental age in fig. 2.

(As will be seen from the argument, the actual values for the vertical scale cannot be given, but this does not affect the subsequent argument.)

The fact that the correlation of this ratio with mental age is significant and positive clearly invites interpretation. As we pointed out above, these two constituents clearly correspond to two natural parts of a cell—the nucleus and the cytoplasm. Consequently we may first draw the provisional conclusion that *on the average, as one goes from 0 to 14 years of mental age, the*

total amount of nuclear substance in the cortex remains about the same while the amount of cytoplasmic substance increases. This we consider to be little more than a plain statement of fact. It is difficult to think of any other conceivable alternative.

To carry the interpretation a stage further, we are of the opinion that there are two main possibilities :

(a) In the development of the brain, the neuroblasts are at first small cells which consist mostly of nucleus with but little cytoplasm. As growth continues, the cells throw out more processes and thereby become relatively less nucleated. Consequently, we can see that our results, in a general way, suggest that the nerve-cells of the defective's cortex correspond, in their relatively diminished cytoplasm, with cells which are only partially developed. We may note also, that if the defective's nerve-cells are held to be immature, then our work suggests that the immaturity shows itself chiefly in the lack of cytoplasm rather than in any alterations in the amount of nuclear material.

(b) An alternative explanation which may fit the facts is given by considering the neuroglia. Although there are no accurate measurements available, yet a general histological study of the neuroglia suggests that, on the whole, it tends to have less cytoplasm than the average nerve-cell. Consequently our finding that the defective's cortex is relatively rich in nucleoprotein might be due to an increased proportion of neuroglia in the defectives. The question whether defectives' brains differ from the normal in the amount of neuroglia does not appear to have been systematically investigated, and consequently we cannot decide at present between the two possibilities (a) and (b). Of course, the two possibilities are not necessarily exclusive ; it is possible that both occur.

To sum up our conclusions with regard to the nucleoprotein figures, our findings suggest that either the nerve-cells of the defective's cortex are immature in form, or that there is an excess of neuroglia present.

SUMMARY

1. We (A.G.) have analysed chemically the grey matter of the frontal lobe of 71 brains, 62 being from mental defectives of varying mental ages and 9 being from normal adults.

2. The cortex has been analysed into : water, total extract, residue, cholesterol, cerebrosides, phosphatides, and protein-phosphorus.

3. In each method the experimental error has been measured and is below certain limits.

4. The brains being fixed in formalin, the conclusions are subject to certain reservations which are discussed.

5. On testing for any alterations with mental age, we find :

(a) The water content shows a significant correlation with mental age, the normal brains being drier than the defectives'.

(b) The myelin content shows no change with mental age.

- (c) The constituent lipoids—cholesterol, cerebrosides, and phosphatides—show no change with mental age.
- (d) The protein-phosphorus shows a significant correlation with mental age, the defective's brains being relatively richer in protein-phosphorus than the normals.
- (e) Reasons are given for deducing that in the defective either the nerve-cells are immature in form or there is an excess of neuroglia present.

REFERENCES

- ¹ ASHBY, W. R., and STEWART, R. M. (In the press.)
- ² BACKLIN, E., *Upsala Lak. Forhandl.*, 1930, **35**, 105.
- ³ BANG, I., *Mikromethoden der Blutanalyse*, 1922.
- ⁴ DONALDSON, H. H., *Jour. Comp. Neurol. and Psychol.*, 1910, **20**, 119.
- ⁵ FISHER, R. A., *Statistical Methods for Research Workers*, 1925.
- ⁶ FISKE, C. H., and SUBBAROW, Y., *Jour. Biol. Chem.*, 1925, **66**, 375.
- ⁷ GORODISSKAY, H., *Biochem. Zeits.*, 1925, **159**, 379 ; 1925, **164**, 446.
- ⁸ GRIGAUT, C. R. *soc. biol.*, 1910, **68**, 791.
- ⁹ KOCH, M. L., and KOCH, W., *Amer. Jour. Physiol.*, 1904, **11**.
- ¹⁰ KOCH, M. L., and KOCH, W., *Jour. Biol. Chem.*, 1913, **14**, 281.
- ¹¹ KOCH, W., and MANN, S. A., *Jour. of Physiol.*, 1907, **36**.
- ¹² KOCH, W., and MANN, S. A., *Arch. Neurol. and Psychiat.*, 1920, **4**, 174.
- ¹³ MACLEAN, H., *Biochem. Jour.*, 1914, **8**, 453.
- ¹⁴ SMITH, J. L., and MAIR, W., *Jour. Pathol. and Bacteriol.*, 1912, **17**, 418.
- ¹⁵ SCHIFF, E., and STRANSKY, E., *Jahrb. f. Kinderheilk.*, 1921, **86**, 245.
- ¹⁶ TILNEY, F., and ROSETT, J., *Bull. Neurol. Inst. New York*, 1931, **1**, 28.