BLOOD AMINES.

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IN the normal individual toxic amines produced by bacterial action in the gut or elsewhere are readily destroyed by the amine oxidase, an enzyme which is present in the liver, intestine and other organs (Richter, 1938; Blaschko, Richter and Schlossmann, 1937). This process of detoxication is normally very rapid and efficient, but it has been suggested that in certain pathological conditions the detoxicating system may be defective, giving rise to an abnormally high level of toxic amines in the blood (Quastel, 1937).

The idea of autointoxication by amines has been current for many years, and it has frequently been suggested as a possible causative factor in mental disease (Alvarez, 1924; Quastel, 1932; Buscaino, 1928); but no direct estimations of the amount of amines in the blood in pathological conditions have hitherto been made, nor in fact have suitable methods for such an investigation been available.

Advantage has now been taken of a new and very sensitive method of estimating blood amines (Richter, Lee and Hill, 1941) to make a preliminary investigation of the amines in the blood in a group of 58 selected psychotics. The psychotics were especially selected from a group of 2,000 available patients as being typical cases of the psychoses diagnosed and free from other complicating mental or physical disorders. Specimens of venous blood were tested for (*a*) simple amines of the β-phenylethylamine or isoamylamine types and (*b*) free "amino-lipids," i.e. lipids containing an amino-group and free in the sense of being extractable from aqueous solution by petroleum ether under the conditions described.

METHODS.

The blood samples (10 ml.) were collected about noon in tubes containing potassium oxalate as anti-coagulant. The amine estimations were carried out within a few hours of collecting the blood, and the methods of estimation were similar to those described by Richter, Lee and Hill (1941); the amines were extracted from the blood (10 ml.) by shaking for

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three minutes with petroleum ether (7 ml.) and an excess of saturated potassium carbonate solution (5 ml.), which liberates the free amines from their salts and at the same time exerts a salting-out action. The emulsion which often formed was broken down by alternately centrifuging and stirring in the centrifuge tube with a thick glass rod. The amines were separated from the lipids by shaking 3 ml. of the petroleum ether extract with 0·5 ml. of a solution of N/1 sulphuric acid which had been saturated with sodium bromide ; this solution dissolves the simple amines but not the lipids. After washing the acid solution with 3 ml. fresh petroleum ether, which was discarded, 0·4 ml. of the acid solution was pipetted into a clean centrifuge tube and 2·4 ml. petroleum ether and 0·2 ml. 40 per cent. potassium hydroxide were added. The mixture was shaken, centrifuged to break the emulsion, and the petroleum ether layer transferred to a clean dry test tube. Chloroform (2·4 ml.) was added and, after shaking up, 0·1 ml. 2 per cent. picric acid in chloroform was added, to form the yellow picrates of any amines present. The yellow colour was then compared with a series of standard solutions which had been prepared by treating aqueous solutions of β-phenylethylamine containing 0·5 to 10 μg./ml. in exactly the same way as the blood. A blank test was always done at the same time as each series of blood samples on distilled water. Further experimental details of the method are given by Richter, Lee and Hill (1941). It was found by carrying out the test on samples of normal blood to which different amounts of β-phenylethylamine had been added that a concentration of 8 μg./ml. or 8 parts per million of β-phenylethylamine could be distinguished with certainty from the blank to which none had been added ; this was therefore the limit of the sensitivity of the method under these conditions.

The estimation of free " amino-lipids " was done by mixing 1 ml. chloroform with 1 ml. of the first petroleum ether solution after extraction with NaBr : H_2SO_4 and shaking with 0·05 ml. of 2 per cent. picric acid in chloroform. The yellow colour was then compared with a series of standard solutions prepared in the same way from aqueous β-phenylethylamine solutions, which served as an arbitrary scale for comparison. The limit of sensitivity of this estimation, which involved fewer operations than the simple amine estimation, corresponded to 0·5 μg./ml. of β-phenylethylamine. The substances in blood which give this reaction have not yet been clearly identified (Kirk, 1940) ; but the reaction is given by any lipid extracted by petroleum ether and containing an amino-group, so that it includes free phosphatides extracted under these conditions, which react by reason of the primary or quaternary amino-groups in the choline or ethanolamine residues.

Results.

The tests for simple amines were negative in all of the 58 patients examined. That is to say that all of the specimens tested contained less than 8 parts

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per million of β-phenylethylamine or of the higher aliphatic amines which are formed by the putrefactive bacteria.

The free "amino-lipids" in the blood varied in amount from 0 to 7, expressed as the concentration of β-phenylethylamine (μg./ml.) which gave an equivalent colour intensity. None of the groups of psychotics showed any very striking difference in "amino-lipid" content, but it was noticeable that schizophrenics generally gave values that were higher than the others. The average value for the 18 schizophrenics was 1·76 (standard deviation 2·73), compared with the mean of 0·95 (standard deviation 0·81) for the other 37 patients tested. The difference in the means was 85 per cent. Examined statistically by Fisher's *t* test (Fisher, 1932), which is specially designed to test the significance of a difference of the means in small groups, *t* came to 2·267; this indicates that the probability is approximately 30 to 1 that the higher values found for amino-lipids in the schizophrenic group are real and not due to chance.

The figures obtained for the different groups of psychotics were as follows:

Diagnosis.	Amines. Number of cases.	Amines. Amount found.	"Amino-lipids." Number of cases.	"Amino-lipids." Amounts found.
Manic-depressive psychosis:				
Depressed phase	16	0	15	1; 0·5; 0·8; 2; 0·8; 0·7; 2; 1; 3; 0·5; 0·8; 1; 0·8; 1; 0·5.
Intermediate phase	4	0	4	0·7; 0·8; 1; 0·7.
Manic phase	2	0	2	0·8; 1.
Schizophrenia: Recent acute	2	0	2	1; 5.
,, Chronic	17	0	16	1; 7; 0·8; 0; 2; 2; 0·7; 2; 1; 2; 1; 1; 0·6; 2; 2; 0·6.
Paranoia	3	0	2	0·5; 0·7.
Epileptic psychosis	6	0	6	0; 0·5; 0·6; 0; 0·5; 0·6.
G.P.I.	3	0	2	5; 0·8.
Post-encephalitis	5	0	5	0·6; 0; 2; 0·9; 0.

("Amino-lipid" content for schizophrenics: Mean 1·76; σ 2·73.
,, ,, other psychotics: Mean 0·95; σ 0·81.)

Discussion.

The idea of autointoxication by toxic substances, produced by bacteria in the intestine or through some metabolic defect in the body, is one of the oldest theories of mental disease. Watson (1923) has reviewed the earlier literature on the subject. The particular interest in amines derives mainly from the classical work of Barger and Dale, who first investigated the physiological properties of a large number of amines and demonstrated by isolation that they are formed in putrefactive processes. The production of schizophrenic symptoms by the amine mescaline (Guttmann and Maclay, 1936) and the

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observation that amines inhibit the respiration of brain slices *in vitro* (Quastel and Wheatley, 1933) have emphasized the possibility that toxic amines might be concerned in the causation of mental disease.

Looney (1924) found that the " undetermined N " in the blood was higher in depressed psychotics and in certain types of dementia praecox than in the normal. The mean " undetermined N " in 26 depressives was 7·75 mgm.N./100 ml. compared with a mean of 5·73 in a group of 19 normals. He concluded that " evidence has been produced pointing to the view that toxic amines are present in the blood of markedly depressed cases. . . . Absolute proof that such amines are associated with depressions cannot, of course, be given without first acquiring some method for isolating them and measuring them quantitatively." Since the normal figures for " undetermined N " varied from 0·32 to 10·90, the difference of 2·02 in the means for the normals and depressives might appear to be of dubious significance ; but when Looney's figures are examined statistically the significance, expressed as *t*, comes to 2·08, or the probability is 22 to 1 that the difference is not due to chance. Taken together with Looney's further figures for a smaller group of involutional melancholics (mean 8·58) and the independent confirmation by Reid (1927), the figures indicate a considerable probability that the blood of depressed psychotics contains an abnormal amount of an unidentified nitrogenous compound in the " undetermined N " fraction. This work provided no evidence, however, that this substance (*a*) is toxic, (*b*) is an amine or (*c*) is in any way related to the mental condition. In this connection Buscaino's " black " reaction, which he attributed to a toxic amine in the urine of psychotics (Buscaino, 1923), but which was finally shown to be due to their diet and not their mental condition (Mann and Shipp, 1929 ; Katzenelbogen, 1929), gives a warning against the too facile assumption that an abnormality in the blood is necessarily related to the mental state.

Stewart (1929) attempted to obtain evidence on the amine intoxication hypothesis by looking for the degradation products of amines in the urine, and he reported a considerable increase in these products in the urine of a small group of psychotics. This observation might appear suggestive, but it may be doubted whether it is due to anything more than an increased tendency to constipation in the psychotics and a consequently increased breakdown of proteins by the intestinal bacteria.

The recent work of Gjessing (1939) has renewed interest in the question of toxic amines, since he has shown that the phasic changes in the mental state of periodic catatonics are associated with phasic changes in the nitrogen excretion, and he has 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 (this work has been reviewed by Stokes, 1939). There are difficulties, however, in applying this hypothesis, since during the time of mental disturbance some patients are found to be in a state of positive nitrogen

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balance, while in others the nitrogen balance is negative. The retention of nitrogen observed in these cases applies to all the protein degradation products found in the urine, which suggests that the fundamental metabolic disturbance involves the whole protein catabolism, and not merely the metabolism of a single protein intermediate, which might lead to the production of a toxic amine. It may also be questioned whether the metabolic disturbance is limited to the nitrogen metabolism, and whether further investigation may not show that the carbohydrate and lipid metabolism are also affected. Gjessing's work gives only very indirect evidence of the possibility that toxic amines might be involved.

The most suggestive evidence in this field is still Looney's observation of an increased amount of an unidentified nitrogenous compound in the blood of depressives. In testing for the presence of amines two types of compound appeared of particular interest: (*a*) The simple aliphatic amines and their derivatives, such as β-phenylethylamine, which are known to be formed by the putrefactive bacteria in the intestine, and (*b*) "amino-lipids," or lipids containing a basic N-atom, compounds which are important constituents of nervous tissue.

There is no evidence from which can be estimated the concentration of amines that would need to be present in the blood for the symptoms observed in the psychoses to be produced. Doses up to 300 mgm. of β-phenylethylamine taken by one of us (D. R., 70 kgm.) by mouth produced definite subjective mental symptoms, a feeling of anxious depression and a feeling of unreality, as well as physiological effects on the blood pressure and heart rate and a severe headache. With this dose the concentration in the blood could not exceed 60 μg./ml. and was probably much lower; but it has been shown that the body soon adapts itself to the administration of amines so that subsequent doses produce much less effect than the first (Dale and Dixon, 1909), and a much higher level would therefore be needed in the blood to produce an effect lasting over a considerable period of time. In some of his depressed cases Looney obtained "undetermined N" figures as high as 14·2 mgm. N./100 ml., which would correspond to a level of over 1,000 μg./ml. of an amine such as β-phenylethylamine. The methods used in the present investigation were sensitive enough to detect concentrations as low as 8 μg./ml. of β-phenylethylamine; but apart from a slight increase in the amino-lipid fraction in the schizophrenics no significant amount of amines could be detected in the blood of any of the 58 psychotics examined. A "flooding of the system" with amines, such as Stewart suggested, can be excluded for amines of the two types described.

The increased "amino-lipid" fraction found in the schizophrenics is clearly unrelated to Looney's "undetermined N," which was raised in a different class of psychotic. Since the fraction in which the increase occurred includes the free phosphatides, it appears more likely that it is due to a nutritional abnormality than that it is connected with their mental state, but if this

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finding can be repeated in other groups of schizophrenics it might serve as a useful indication for further research.

Amines are known to produce symptoms which are similar to those observed in psychotics, but almost any foreign chemical substance may interfere with the normal working of the brain, and this property is by no means limited to amines. Mental symptoms are produced, for example, by the lower and higher alcohols, esters, aldehydes and glycosides as well as by a variety of nitrogenous compounds of many different types, such as urethanes, hydantoin derivatives, barbiturates, triazoles, nitrites and numerous alkaloids. Why then, it may be asked, has so much emphasis been laid on the possibility of autointoxication by amines? Apart from the very indirect and circumstantial evidence already mentioned we have been unable to find any adequate evidence in favour of the toxic amine hypothesis. It has recently been shown that amines which are not detoxicated by the amine oxidase can still be removed from the system by concentration in the kidney and elimination in the urine (Richter, 1938), and this would appear to make the hypothesis still less probable. A method of testing the amine oxidase function is now available (Richter, Lee and Hill, 1941), and it would seem that unless more definite clinical evidence, such as a defect in amine oxidase function, can be demonstrated in some clearly defined group of psychotics, the toxic amine hypothesis should no longer be regarded as anything more than a speculation.

Summary.

Sensitive tests for (*a*) alkylamines and their derivatives and (*b*) "amino-lipids," or lipids containing a basic N-atom, have been applied to the blood of 58 selected psychotics of different types. In every case the blood contained less than 8 parts per million of amines of the β-phenylethylamine or isoamylamine types. A slight increase in the free "amino-lipid" fraction was noted in the schizophrenics. Statistical analysis of the figures indicates that the probability is 30 to 1 that the observed increase in the "amino-lipid" fraction in the schizophrenics is not due to chance. The basis of the toxic amine hypothesis has been discussed.

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