VARIATIONS IN THE BLOOD LEVELS OF ACETOIN AND BUTANE-2:3-DIOL IN NORMAL INDIVIDUALS AND MENTAL PATIENTS

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(Received 9 October, 1953)

THE biochemical aspects of manic-depressive psychosis have been adequately reviewed by McFarland and Goldstein (1939). From previous investigations, it is apparent that there is a marked alteration in carbohydrate metabolism mainly associated with a change in glucose tolerance. This phenomenon is related to alterations in emotional state and there is no suggestion that it is the cause of the mood swings which occur periodically in this type of psychosis. Apart from these variations in the control of carbohydrate metabolism, it has so far not been possible to demonstrate any changes in the blood levels of any products derived from carbohydrate metabolism.

During investigations in cases of *diabetes mellitus* in which the control of the disease was inadequate, it was found that the blood acetoin (AMC) level was raised. In view of the decreased glucose tolerance observed during the depressed phase of manic-depressive psychosis by McCowan and Quastel (1931), Hackfield (1932), Northcote (1932), and Ström-Olsen (1932), it was thought to be of interest to investigate variations in the AMC and butane-2:3-diol (2:3BG) blood levels during the mood phases of this mental disorder. In addition, a similar investigation was carried out on a heterogeneous collection of psychoneurotic individuals.

In animals, Stotz, Westerfeld and Berg (1944) demonstrated that acetaldehyde, when injected intravenously, produced a rise in the blood level of AMC whilst pyruvate and a mixture of pyruvate and ethyl alcohol did not. They were of the opinion that AMC was rapidly metabolized, thus differing from Greenberg (1942) who showed a slow rate of disappearance of injected *dl*-acetoin. Dawson and Hullin (1952) have demonstrated that intravenous pyruvate injections in decerebrated cats will produce a rise in AMC blood level but their results agree with Stotz *et al.* in that injected AMC in similar preparations is rapidly metabolized.

In micro-organisms, AMC and 2:3BG can be produced from pyruvate and glucose, the relative amounts of the two compounds produced depending on the substrate used as shown by Happold and Spencer (1952). Watt and Krampitz (1947) and, more recently, Juni (1950) have shown that AMC is produced from pyruvate by the following mechanism.

Pyruvate + acetaldehyde ——→ α-acetolactic acid ——→ AMC

Experimental

Clinical material

The mental patients studied in this survey were at the Retreat, York, and were under the care of one of us (A.P.) who was responsible for their selection and classification.

Procedure adopted in collecting the blood samples

Except for the results shown in Table I where sodium citrate was used as an anti-coagulant, the blood collected intravenously was immediately ejected from the syringe into a graduated centrifuge tube containing 10 ml. cold (10 per cent. w/v) trichloroacetic acid and the mixture thoroughly shaken. All samples were assayed as quickly as possible afterwards but were kept at 0° during the intervening period.

Analytical methods

For the results shown in Table I, AMC was estimated by oxidizing it to diacetyl under the conditions used by Stotz and Raborg (1943) and then determining the diacetyl according to the method of White, Krampitz and Werkman (1945). All other estimations for AMC and 2:3BG were carried out using the method of Happold and Spencer (1952), slightly modified as follows for use on blood samples.

The accurate volume of the blood taken (ca. 10 ml.) was measured in the graduated tube after centrifuging for 20 minutes. The supernatant solution was then decanted and the precipitated blood proteins re-extracted by stirring with more cold trichloroacetic acid (12 ml.). After further centrifuging, the supernatant solutions were combined and the volume of acid extract noted; preliminary experiments had shown that all the carbinol and glycol was removed by the two extractions.

AMC was assayed on an aliquot portion of the acid extract. The total of carbinol and glycol present was determined on a 6 ml. portion of the extract which was first adjusted to pH 7 by addition of sodium hydroxide solution (approximately 0·15 ml. of 40 per cent. (w/v) NaOH solution was required), the neutralization being completed by dropwise addition of 10 per cent. (w/v) NaOH solution, using bromothymol blue as external indicator.

The precipitated hydrazone was washed with 5 ml. of 5 per cent. (w/v) aqueous methyl alcohol to eliminate any possible interference from glucose in the blood filtrate.

Recovery experiments indicated that the method of Happold and Spencer as modified for blood samples was accurate to within ±5 per cent. and concentrations as low as 15 μg./100 ml. of blood could be determined.

Table I

Variations in the blood AMC level in different mental states

Mental State	Subjects	Blood AMC (μg./100 ml.) Mean	±	S.E.M.
Normal	32	31	±	2·3
Depressed	46	50	±	8·2
Manic	35	49	±	7·7
Psychoneurotic ..	17	28	±	5·1

https://doi.org/10.1192/bjp.100.419.536 Published online by Cambridge University Press

Results

The clinical investigation was divided into four different attempts to determine whether a variation in AMC and 2:3BG blood levels could be correlated with mood swings in manic-depressive psychosis.

1. This was an investigation into the blood AMC level in a large series of mental patients who were in various stages of depression and mania but who had not received either electro-convulsive therapy or insulin shock treatment. A group of patients suffering from various psychoneuroses was also investigated.

The difference between the AMC level of the manic and depressed phases and that of normal individuals and psychoneurotics is just significant ($p<0\cdot05$).

2. Using the more sensitive, Happold and Spencer method for AMC determinations and including, in addition, 2:3BG estimations, a further series of new admissions to the Retreat was investigated before E.C.T. or insulin shock therapy had been instituted. The normals were volunteers from the staff of the hospital.

Table II

Variations in the blood AMC and 2:3BG level in certain mental states

Mental State	Subjects	Blood AMC (μg./100 ml.) Mean	±	S.E.M.	Blood 2:3BG (μg./100 ml.) Mean	±	S.E.M.
Normal	13	49	±	7·0	232	±	13·6
Depressed	42	73	±	8·7	237	±	18·6
Manic	6	85	±	18·1	406	±	105·9

The increase in blood AMC level above the normal range in both the depressed and manic phases is just significant ($p<0\cdot05$). The 2:3BG level in the depressed phase is within normal limits and, in the manic phase, is too variable to draw any conclusions from the small number of individuals studied.

3. The previous two studies had been carried out on blood obtained during the absorptive phase after a meal (the samples had been taken within 30 minutes after breakfast). In view of the rather wide variation in results obtained, blood taken in the fasting state before breakfast was used for subsequent investigations. In addition, treatment by sedation for the 48 hours prior to blood sampling was discontinued. No other form of physical treatment had been instituted as in the previous surveys.

Table III

Variations in the fasting blood level of AMC and 2:3BG in cases of depression

Mental State	Subjects	Blood AMC (μg./100 ml.) Mean	±	S.E.M.	Blood 2:3BG (μg./100 ml.) Mean	±	S.E.M.
Normal	22	74	±	6·9	175	±	13·1
Depressed	22	148	±	18·7	204	±	26·2

In normal individuals the blood AMC level is increased in the fasting state ($p<0\cdot025$) whilst the 2:3BG shows a significant decrease ($p<0\cdot01$) as compared with the non-fasting state (compare Table II).

The fasting blood AMC level is significantly increased in the depressed phase ($p<0\cdot002$) without any significant change in the 2:3BG level.

4. This investigation concerns two individual patients (one male, one female) with a type of manic-depressive psychosis showing rapid fluctuations

TABLE IV

Variations in the fasting blood concentrations of AMC and 2:3BG in manic-depressive psychosis

Mental State	No. of estimations	Blood AMC concentration (μg./100 ml.) Mean	±	S.E.M.	Blood 2:3BG concentration (μg./100 ml.) Mean	±	S.E.M.	Total concentration of AMC+2:3BG (μg./100 ml.) Mean	±	S.E.M.	2:3BG AMC ratio
Normal group	30	83	±	4·7	194	±	6·6	277	±	5·7	2·3
Normal phases of psychotic group	11	89	±	10·6	168	±	19·3	257	±	15·0	1·9
Depressed phases of psychotic group	17	111	±	13·8	223	±	21·3	334	±	17·7	2·0
Manic phases of psychotic group	17	52	±	7·8	284	±	14·1	336	±	11·0	5·5

in mood swing. Blood levels of AMC and 2:3BG were determined in the fasting state at twice-weekly intervals and correlated with the mood swings. In both cases, all physical treatment (including the use of sedatives) was withheld during the periods of the survey. The results embrace two separate surveys on each patient at an interval of 3 months; each survey lasted 5-6 weeks. Table IV represents the mean values obtained over a considerable number of mood swings; included in the table are values obtained in a similar way for a group of 3 normal individuals.

During the normal phase of the psychosis, the blood levels of AMC and 2:3BG do not differ significantly from the control group. The blood AMC level in the depressed phase is significantly raised above that of the control group ($p<0\cdot02$); it is not significantly raised above the level found during the periods of normality of the psychotics. The 2:3BG levels are not significantly different from the normal level.

In the manic phase, the blood carbinol level is reduced below the level of both the control group ($p<0\cdot002$) and the normal phases of the psychosis ($p<0\cdot01$). The 2:3BG levels in the manic phase are significantly higher than either of the two normal groups ($p<0\cdot002$).

The 2:3BG-AMC ratio is not significantly altered in depression but is markedly increased in mania. The total concentration of carbinol and glycol is significantly increased in both the manic and depressed phases ($p<0\cdot025$).

5. The effect of intense physical activity on 5 normal subjects was studied by means of a cycle ergometer. The test was carried out before breakfast. A fasting blood sample was taken and the subject then vigorously exercised for 20-30 minutes against a 3·5 kilogram load of the ergometer. Within 2 minutes of the completion of the exercise a second blood sample was taken as previously and both samples assayed for pyruvate, AMC and 2:3BG.

TABLE V

The effect of vigorous exercise on the blood levels of AMC, 2:3BG and pyruvate in five normal fasting subjects

	Before exercise			After exercise		
	Mean	±	S.E.M.	Mean	±	S.E.M.
AMC (μg./100 ml.) ..	63·2	±	10·0	65·6	±	22·7
2:3BG (μg./100 ml.) ..	183·4	±	46·7	196·2	±	25·0
Pyruvate (mg.%) ..	1·44	±	0·32	2·21	±	0·39

There was no significant difference between the blood concentrations of AMC and 2:3BG before and after exercise. The increase in pyruvate level after exercise was characteristic of changes associated with strenuous exercise.

DISCUSSION

Acetoin and butane-2:3-diol are normally present in blood, the amount varying within narrow limits in normal individuals, with higher carbinol levels in the fasting state. From the results of previous workers, pyruvate would appear to be the main source of AMC although acetaldehyde could be a precursor in combination with pyruvate. The rate of production *in vivo* is unknown and AMC formation would appear to be a side reaction as far as pyruvate metabolism is concerned.

In the cases of manic-depressive psychosis, two features concerning AMC are apparent; the extreme variability of the blood levels and the rise which

occurs in the depressed phase. This rise could either be a manifestation of an over-production or of a decreased utilization and is not related, in the depressed phase, to an altered equilibrium state between AMC and 2:3BG since the ratio of the levels of the two compounds is within normal limits.

In the manic phase, however, although there is an increase in the total amount of carbinol and glycol produced, there is also a change in the relative amounts of these compounds. Thus, manic-depressive psychosis in the fasting state is characterized not only by an increased total of AMC and 2:3BG in the blood, but in the manic phase, an increased amount exists as 2:3BG. In mania, the AMC concentration in the non-fasting state is increased whilst it is significantly lower than normal in the fasting state. This is the reverse of the change observed in normal individuals where the carbinol level is greater in the fasting state than in the non-fasting state. Thus the alteration in metabolism involves not only variations in either the rate of production or utilization, but also variations in the amount of reduction of AMC at the equilibrium point.

Manic-depressive psychosis is a disease entity which embraces alterations in mental state together with profound changes in physical activity; the physical and mental symptoms are so inter-woven that it seems impossible to separate them—indeed it would not be justifiable to attempt to do so in any consideration of the whole disorder.

In the mentally normal subjects, intense, prolonged physical activity produces no significant alteration in AMC and 2:3BG blood levels. It is, however, doubtful whether even the most intense activity which can be provoked in normal individuals can be compared with the activity observed in the manic phases of manic-depressive psychosis. This may range in the latter case from rapid changes of ideas and flights of fancy, to intense, frenzied physical activity leading to exhaustion. Attempts to exclude the possibility that the physical manifestations are responsible for alterations in blood chemistry are likely to be abortive owing to the difficulty of simulating such activities in normal individuals. Consequently we have regarded manic-depressive psychosis as an entity and used normal subjects behaving in a normal manner as controls. This means, of course, that at this stage of the investigation we cannot conclude whether the changes observed are due to the altered mental state interfering with metabolic activity, whether the altered physical activity is responsible, or whether the metabolic alteration leads to a change in mental function. In view of the widespread distribution in the body of the enzymic mechanism for producing AMC no indication concerning the site of the alteration can be inferred from this consideration.

Since fasting alone will change the level of carbinol in the blood, it would seem that AMC production (or its rate of destruction) is governed by some controlling mechanism and it may be that, in cases of manic-depressive psychosis, this controlling mechanism is disturbed.

The results obtained indicate that a metabolic disturbance does occur in manic-depressive psychosis and is peculiar to the psychosis in so far as it shows a periodicity which can be correlated with the mood swings; it cannot be simulated in normal people by alterations in physical activity.

SUMMARY

1. In the non-fasting state the blood acetoin (AMC) level of a group of mental patients in the manic and depressed phases was higher than that of a normal group or of a group of psychoneurotic individuals: the degree of variation in blood level was also greater in the psychotic group.

2. In normal individuals, the blood AMC level in the fasting state is raised and the butane-2:3-diol (2:3BG) level reduced as compared with the levels in the non-fasting state.

3. In the fasting state, manic-depressive psychosis is characterized by an increased total concentration of AMC and 2:3BG in the blood. In the depressed phases of the psychosis, the carbinol level is raised but no significant change in the glycol concentration occurs. In the manic phases, the blood carbinol level is reduced whilst the glycol level is greater than normal. These variations produce a marked change in the 2:3BG-AMC ratio in the manic phase.

Acknowledgments

The authors acknowledge the very helpful co-operation of Dr. C. C. Beresford, the medical and nursing staff, and also the invaluable technical assistance of Mrs. D. Oliver, of the Retreat, York. We should also like to thank all those who volunteered as subjects for the normal group, and in particular A. R. Johnson and R. L. Noble of the Biochemistry Department. Our thanks are also due to Professor F. C. Happold for much helpful advice and stimulating criticism and to the Committee of Management of the Retreat for a grant in aid of this work.

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