Brit. J. Psychiat. (1963), **109**, 231–234

The Effect of Plasma from Schizophrenic Patients on the Chicken Erythrocyte System

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Frohman *et al.* (1960a, 1960b, 1961, 1962) have investigated the effect of plasma of schizophrenic patients on carbohydrate metabolism. They measured the production of lactate and pyruvate by the nucleated red cells of the chicken after incubation with patients' plasma. After incubation with plasma from schizophrenic patients, the lactate/pyruvate ratio was raised significantly above the ratio found after incubation with plasma from normal subjects. This observation was interpreted as evidence of an inhibition of aerobic metabolism induced by the plasma of schizophrenic patients. We report here an attempt to confirm these findings.

Methods

Seven chronic male schizophrenic patients and ten normal men were chosen for this study. The mean age of the patients was 39 years (range 19–57) and that of the controls was 33 years (range 22–46). The patients had been at St. Ebba's Hospital for periods ranging from 3 weeks to 23 years. Care was taken to select patients in whom the diagnosis had been agreed on by at least three psychiatrists and regarded as unequivocal. None of the patients was known to have had symptoms of schizophrenia in childhood. Four patients had not been taking any drug for at least a month, three were taking paraldehyde or chloral hydrate at night. No substantial difference was observed in the results between these latter patients and those who were taking no medication at all. The diet for the patients was good and was generally similar to that of the controls, who were on the staff of the hospital. All subjects were free from physical disorders at the time of the investigation. Initially controls and patients were engaged in their everyday activities and were rested for 10 minutes before blood was collected. In the last two experiments subjects were rested in bed for half an hour before the blood was collected. This made no apparent difference to the results and these were pooled with the others. Each patient was paired with a control and blood was taken from patient and control within a few minutes of each other. All subjects were fasted for 12 hours before blood samples were taken. Blood was collected in heparinized syringes, following the procedure recommended by Friedemann and Haugen (1943) for the determination of blood pyruvate, and was immediately centrifuged. The plasma was transferred to iced tubes within one hour after collection of the blood and was kept in ice until the experiment, which was performed on the same day. At this stage the plasmas were coded and the estimations were carried out "blindly".

Four Light-Sussex chickens were used throughout the experiment, each bird being used at four-week intervals. Blood (12–13 ml.) was drawn from a wing vein into a syringe containing 3 ml. of 3·2 per cent. sodium citrate: the blood was immediately centrifuged at +1° C. at 1,200 g. for 10 minutes. Plasma was removed and the cells were washed once with three volumes of cold 0·9 per cent. NaCl solution; the cells were again centrifuged for 10 minutes at the same speed. The saline was then removed and the cells were suspended in one volume of Krebs-Ringer phosphate buffer (Krebs, 1933) with 0·075 M glucose ($CaCl_2$ was omitted in all buffers used in our experiments).

Incubation was started four to seven hours after the chicken blood was collected. The cell suspension (1·32 ml.) was added to 0·5 ml. of plasma from either a schizophrenic or a control subject and to 1·18 ml. of Krebs-Ringer bi-

carbonate buffer (Krebs and Henseleit, 1932). The final concentration of inorganic phosphate in the incubation medium was 4·7 mM. At the end of the incubation period, 0·1 ml. of the incubation mixture was transferred into a test tube containing 1·9 ml. of isotonic saline, which was then gently dispersed and centrifuged. Haemoglobin in the supernatant fluid and in the sediment was determined as acid haematin in a Unicam 600 spectrophotometer (wave length 380 mμ). The haemoglobin of the supernatant provided an index of the amount of haemolysis.

Incubation blanks were prepared by replacing 1·32 ml. of cell suspension with 1·32 ml. of a mixture of 1 volume of 0·9 per cent. NaCl and 1 volume of the Krebs-Ringer medium. Experimental samples and blanks were incubated at 37° C. in a Dubnoff shaking incubator in an atmosphere of 5 per cent. CO_2 in air for 60 minutes. Incubation was stopped with an equal volume of 20 per cent. trichloroacetic acid. In all but two experiments incubation was performed in duplicate and the results were averaged. The coefficient of variation was 5 per cent. for the pyruvate determinations, 6 per cent. for the lactate and 9 per cent. for the lactate/pyruvate ratios.

Lactic acid was determined by the method of Barker and Summerson (1941). Pyruvic acid was determined by the method of Friedemann and Haugen (1943) using benzene and ethyl acetate as solvents and reading the samples at 435 mμ in a Unicam 600 spectrophotometer. In preliminary experiments pyruvic acid was determined also by an enzymatic method, Segal *et al.* (1956) which gave results 2 per cent. lower than the colorimetric method.

Since our buffer differed from that used by Frohman (1960a, 1961), experiments were also carried out with a Krebs-Ringer phosphate buffer similar to that recommended by these authors (final concentration of inorganic phosphate 27·7 mM. and volume of the incubation mixture 2·5 ml. instead of the usual 3 ml.), and with a Krebs-Ringer bicarbonate buffer containing a higher concentration of phosphate (9·5 mM. in the incubation medium).

Results and Discussion

As shown in Table I, there was no significant difference in the mean lactate/pyruvate ratios between schizophrenic and control subjects. There was also no substantial difference between controls and schizophrenics in any individual experiment. Although the mean values of lactate and pyruvate were somewhat higher in the schizophrenic group, these differences are not significant statistically. Our results are at

Table I

The Effect of Normal and Schizophrenic Plasma on the Level of Lactate and Pyruvate in Chicken Erythrocyte Suspension

Figures represent the mean and the standard error (the number of experiments is in parenthesis) after 60 minutes incubation at 37° C. in 5 per cent. CO_2 + air atmosphere

Krebs-Ringer bicarbonate buffer. 4·7 mMolar inorganic phosphate in the incubation medium

Lactate (μgrams/ml. cells*)		Pyruvate (μgrams/ml. cells*)		Lactate/Pyruvate Ratio	
Controls	Schizophrenics	Controls	Schizophrenics	Controls	Schizophrenics
157·5 ± 15·0 (10)	178·3 ± 10·6 (7)	26·6 ± 2·2 (10)	29·8 ± 1·7 (7)	6·44 ± 1·02 (10)	6·00 ± 0·23 (7)

*Determinations in three experiments have shown a concentration of haemoglobin of Gr. 20·50 ± 0·28/100 ml. of the original cell suspension

variance with those reported by Frohman who found a statistically significant difference between the lactate/pyruvate ratios in these two groups. Their groups also differed significantly in the rate of pyruvate production.

Our experimental conditions were similar to those of Frohman and differed only in the buffer used. The bicarbonate buffer was chosen because with the Krebs-Ringer phosphate buffer recommended by Frohman (1960a, 1961) the pH of the incubation medium did not remain constant, but rose from 7·4 to 7·6 in one hour.

However, experiments using the phosphate buffer and a Krebs-Ringer bicarbonate with an increased phosphate concentration showed no difference in lactate/pyruvate ratio between patients and controls (Table II). In the conditions of our experiments we have obtained for all subjects lower values of lactate and pyruvate and higher lactate/pyruvate ratios than those reported by Frohman. This may be due to the different buffer used in our experiments; in fact, in the experiment with a buffer similar to that used by Frohman, our values were closer to those reported by these authors (Table II).

The amount of haemolysis during incubation was estimated. In the experiments reported in Table I, 1·09±0·14 per cent. of the cells were haemolysed.

Frohman *et al.* (1961) have reported that a number of factors can affect the lactate/pyruvate ratios in the chicken erythrocyte system. All of them, except diet, were carefully controlled in our experiments. In our present setting we have been unable to obtain completely similar diets for the two groups of subjects, but there is no reason to suppose that they have differed substantially. Different conditions among subjects of the same experimental group do not seem to have greatly affected our results, as evidenced by the standard errors.

Our chickens were of a different strain from those used in some of Frohman's experiments. We do not know whether this may account for our results. In Frohman's early papers on the subject, the strain of the chickens is not mentioned.

It is unlikely that any difference in clinical material would account for our negative results. Care was taken to include only physically healthy patients in whom the diagnosis was quite certain. None of these patients had suffered from schizophrenic symptoms in childhood, a condition which in Frohman's experiments (Frohman *et al.*, 1962) has shown no difference from the control group.

Summary

Chicken erythrocytes were incubated with plasma from normal and schizophrenic subjects. After one hour's incubation, lactate and pyruvate production were determined. Contrary to previous reports no difference was found in the lactate/pyruvate ratios between the two groups of subjects.

Table II

The Effect of Different Incubation Media on Lactate and Pyruvate Production

Exp. No.	Krebs-Ringer bicarbonate buffer	Krebs-Ringer phosphate buffer	Lactate formed (μgrams/ml. cells)		Pyruvate formed (μgrams/ml. cells)		Lactate/pyruvate ratio	
	Inorganic phosphate mM.		Control	Schiz.	Control	Schiz.	Control	Schiz.
23	4·7	—	162·4	161·3	26·0	28·7	6·24	5·62
	—	27·7	188·9	218·9	47·7	58·2	3·96	3·76
24	4·7	—	197·7	180·7	33·0	29·8	5·99	6·06
	9·5	—	198·9	194·3	39·1	36·0	5·08	5·39

https://doi.org/10.1192/bjp.109.459.231 Published online by Cambridge University Press

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