photometric method[6] and by alkaline hydrolysis. Table 2 summarizes the amino-acid composition of rat growth hormone.

It is of interest that the main component of this rat growth hormone preparation has a molecular weight in the range of that of monkey and human growth hormone and that it lacks the amino-acid tryptophan. The secondary structure of rat growth hormone may well be less complex than that of the growth hormones from other non-primates since it contains only one cystine and three methionine residues per molecule.

R. A. Reisfeld
A. S. Muccilli
D. E. Williams
S. L. Steelman

Merch Sharp and Dohme Research Laboratories,
Division of Merck and Co., Inc.,
Rahway, New Jersey.

[1] Müller-Eberhard, H. J., *Scand. J. Clin. Lab. Invest.*, **12**, 33 (1960).
[2] Reisfeld, R. A., Lewis, U. J., Brink, N. G., and Steelman, S. L., *Endocrinol.*, **71**, 559 (1962).
[3] Sutherland, E. W., Cori, C. F., Haynes, R., and Olsen, N. J., *J. Biol. Chem.*, **180**, 825 (1949).
[4] Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).
[5] Moore, S., *J. Biol. Chem.*, **238**, 235 (1963).
[6] Goodwin, A., and Morton, E., *Biochem. J.*, **40**, 628 (1946).

Failure to find Increased Sodium, Potassium-ATPase in Red Cell Ghosts of Schizophrenics

Seeman and O'Brien[1] have reported that the mean sodium, potassium-ATPase activity in the red cell ghost membranes of 6 schizophrenic patients (79·1 mμmoles ATP split/h/mg dry weight) was significantly greater than in four normal subjects (35·2). Total ATPase was also greater among patients than controls, although the difference in the residual or magnesium-dependent fraction was said to be statistically insignificant. When enzyme activity is expressed in this way, small differences in ghost weight could result in large differences in activity. For this reason it would appear more valid to express sodium, potassium-ATPase as that percentage of the total ATPase activity which is activated by sodium + potassium. When the data for schizophrenic and normal subjects are calculated in this way (sodium, potassium-ATPase × 100/total ATPase), the result for the normals is 16 per cent and for the schizophrenics 24 per cent, a much smaller difference.

It is known that at concentrations above 1×10^{-4} M, strophanthidin completely inhibits the sodium, potassium-ATPase in red cell ghosts and that this degree of inhibition is equivalent to the activation provided by sodium + potassium. We measured the total and strophanthidin-sensitive ATPase activity in red cell ghosts prepared from one sub-acute catatonic and 2 sub-acute paranoid schizophrenics and six healthy controls. None of the patients was receiving drugs. The following method[2] was used to prepare ghosts: fresh cells were hæmolysed in 10 volumes of cold 1×10^{-4} EDTA (ethylenediamine tetraacetic acid) at *p*H 7·4. The resulting ghosts were washed until hæmoglobin-free with a solution containing 0·0153 M sodium chloride, 0·0017 M *tris* and 1×10^{-4} M EDTA (*p*H 7·4). Ghost ATPase activity was determined at 37° C in an incubation solution containing 0·01 M *tris*, 0·002 M sodium ATP, 0·00125 M magnesium chloride, 0·040 M sodium chloride, 0·020 M potassium chloride, and 0·00025 M EDTA (*p*H 7·4) in the presence and absence of $2{\cdot}4 \times 10^{-4}$ M strophanthidin. 1 ml. of ghosts was added to 4 ml. of medium. At 0, 60, and 120 min a portion of the incubation mixture was removed and placed in an equal volume of 6 per cent perchloric acid. Inorganic phosphate was then determined by the method of Berenblum and Chain[3]. Results are given in Table 1 for fresh ghosts and for ghosts frozen at − 20° C for 48 h and thawed prior to the assay. In neither group of ghosts was there a significant difference in total or strophanthidin-sensitive ATPase between normal and schizophrenic subjects.

Examination of the original data of Seeman and O'Brien would be necessary in order to establish whether the difference between normals and schizophrenics in sodium, potassium-ATPase expressed as per cent of total ATPase is statistically significant. If it is, then their finding has not been confirmed by our investigation. The reason that our values, as well as those of others[4,5], for per cent glycoside inhibition are greater than the percentage sodium, potassium-ATPase reported by Seeman and O'Brien is not clear. It may be due to contamination of the preparation with divalent cations or to ageing of the ghosts. This diminished activity has been prevented or counteracted in our studies by the addition of 0·00025 M EDTA to the incubation medium[2].

Table 1. Total and Strophanthidin-Sensitive ATPase in Red Cell Ghosts of Normal and Schizophrenic Subjects

	Phosphate liberation in mμM P_i/mg dried ghosts × h		Strophanthidin inhibition (per cent)
	No strophanthidin	Strophanthidin	
Fresh ghosts			
Normal*	237 ± *S.D.* 22	126 ± 42	46 ± 22
Schizophrenic†	243 ± 74	110 ± 58	51 ± 31
Frozen–thawed ghosts			
Normal*	208 ± 20	87 ± 14	59 ± 21
Schizophrenic†	216 ± 25	58 ± 30	74 ± 11

* One determination on each of five subjects.
† Two determinations on each of two subjects, one determination on a third.

If the sodium–potassium pump of red cells from schizophrenics were different from normals as presumably reflected by differences in sodium, potassium-ATPase activity, it is possible to predict[6] that the sodium and potassium content of the cells would change, *pari passu*, provided that the leak permeabilities did not change in compensation for any changes in pump rate. Thus, for a given leak permeability, the cellular potassium content would increase, and the sodium content would decrease with increasing activity of the pump. Analysis of intact cells showed for normals a sodium content of 10·9 ± *S.D.* 2·0 mM/l. cell water, a potassium content of 146·3 ± 2·4 mM/l., and a water content of 64·7 ± 0·7 per cent; for the schizophrenics, sodium was 12·3 ± 1·3 m.equiv./l., potassium was 152·5 ± 7·2 m.equiv./l., and water content 65·3 ± 1·5 per cent.

The results presented here indicate that if schizophrenics have greater than normal sodium, potassium-ATPase activity in their ghosts, the difference must be a small one, and a large population would have to be studied to establish it.

John C. Parker
Joseph F. Hoffman

Laboratory of Kidney and Electrolyte Metabolism,
National Heart Institute,
Bethesda, Maryland.

[1] Seeman, P. M., and O'Brien, E., *Nature*, **200**, 263 (1963).
[2] Hoffman, J. F., and Ryan, H. E. (unpublished results).
[3] Berenblum, I., and Chain, E., *Biochem. J.*, **32**, 286 (1938).
[4] Post, R. L., Merritt, C. R., Kinsolving, C. R., and Albright, C. D., *J. Biol. Chem.*, **235**, 1796 (1960).
[5] Dunham, E. T., and Glynn, I. M., *J. Physiol.*, **156**, 274 (1961).
[6] Tosteson, D. C., and Hoffman, J. F., *J. Gen. Physiol.*, **44**, 169 (1960).

The findings of Drs. Parker and Hoffman are interesting. Perhaps their smaller differences and our larger differences may be partly explained by a previous administration of tranquillizers. Among our six schizophrenic patients who had elevated sodium-potassium-ATPase values (expressed in absolute units), we found that the highest values were in those who had received phenothiazines during their acute reaction (1–3 weeks) and had then stopped taking drugs about one month prior to the assay of sodium-potassium-ATPase. Since phenothiazines are known to inhibit sodium-potassium-ATPase[1,2], some of the enzyme increase may have resulted as an 'induction' following the period of drug treatment.