

citability of these centers to normal (homeostasis, Darrow and Gellhorn).¹³

The statement by Himwich and Fazekas¹⁴ that my theory of the shock treatment in schizophrenia does not hold since adrenalin is ineffective in the treatment of schizophrenics[†] is obviously based on a gross misunderstanding. The core of the theory (cf. also Gellhorn^{4,6}) is not that insulin coma and electrically- or chemically induced convulsions act through the secretion of adrenalin but that they greatly increase the reactivity of autonomic centers which in turn may influence the brain in such a manner as to restore normal behavior.[‡] The neu-

rological basis for such a hypothalamic-cortical relationship has been elucidated by Murphy and Gellhorn whereas the changes in conditioned reflexes described in this series of papers indicate at least one of the mechanisms which contribute to an altered behavior.

Summary. Conditioned reactions which had been inhibited by lack of reinforcement in rats may be restored by insulin coma in normal as well as in adrenodemedullated rats. Apparently a secretion of adrenalin which is observed under conditions of insulin coma and electroshock does not play an integral part in the mechanism of the restoration of inhibited conditioned reactions. This interpretation is supported by the fact that the injection of adrenalin remains ineffective in the normal animals as far as restoration of inhibited conditioned reactions is concerned, whereas insulin coma and electroshock restore these reactions. It is inferred from these experiments that the restitution of inhibited conditioned reactions is linked up with a hypothalamic-cortical discharge previously established by Murphy and Gellhorn and is not due to the liberation of adrenalin which invariably accompanies the excitation of hypothalamic centers under conditions of insulin coma and electroshock.

¹³ Darrow, C. W., and Gellhorn, E., *Am. J. Physiol.*, 1939, **128**, 185.

¹⁴ Himwich, H. E., and Fazekas, J. F., *Arch. Neurol. and Psychiat.*, 1942, **47**, 800.

[†] The paper by Dynes and Tod¹⁵ to which they refer does not give any evidence for or against the adrenalin treatment of schizophrenics but points out that adrenalin injections cause less emotional reactions (anxiety and fear) in the schizophrenic than in the normal individual.

¹⁵ Dynes, J. D., and Tod, H., *J. Neurol. and Psychiat.*, 1940, **3**, 1.

[‡] The secretion of adrenalin is but one symptom of this central effect and probably of minor importance.

15800

Biological Value of Proteins Determined With *Tetrahymena geleii* H.*

MAX S. DUNN AND LOUIS B. ROCKLAND.

From the Chemical Laboratory, University of California, Los Angeles.

The possibility that the ciliated protozoan, *Tetrahymena geleii* H., might be used to determine the biological value of proteins was

suggested by the observations that this small animal utilizes unhydrolyzed protein¹² and that its amino acid requirements¹³ approx-

* Paper 40. For Paper 39, see Rockland and Dunn.¹ This work was aided by grants from the National Institute of Health and the University of California.

The biological value of proteins has been determined with chickens, dogs, rabbits, rats, swine, and humans by the classical McCollum, Osborne and Mitchell methods as well as by newer technics

described by Cannon *et al.*² and by Harrison and Long.³ Reviews have been given by Mitchell,⁴ Madden and Whipple,⁵ Barnes *et al.*,⁶ Cahill,⁷ Boas-Fixen,^{8,9} Allison *et al.*,¹⁰ and Block and Mitchell.¹¹

¹ Rockland, L. B., and Dunn, M. S., in press (No. 39).

² Cannon, P. P., Humphreys, E. M., Wissler, R. W., and Frazier, L. E., *J. Clin. Invest.*, 1944,

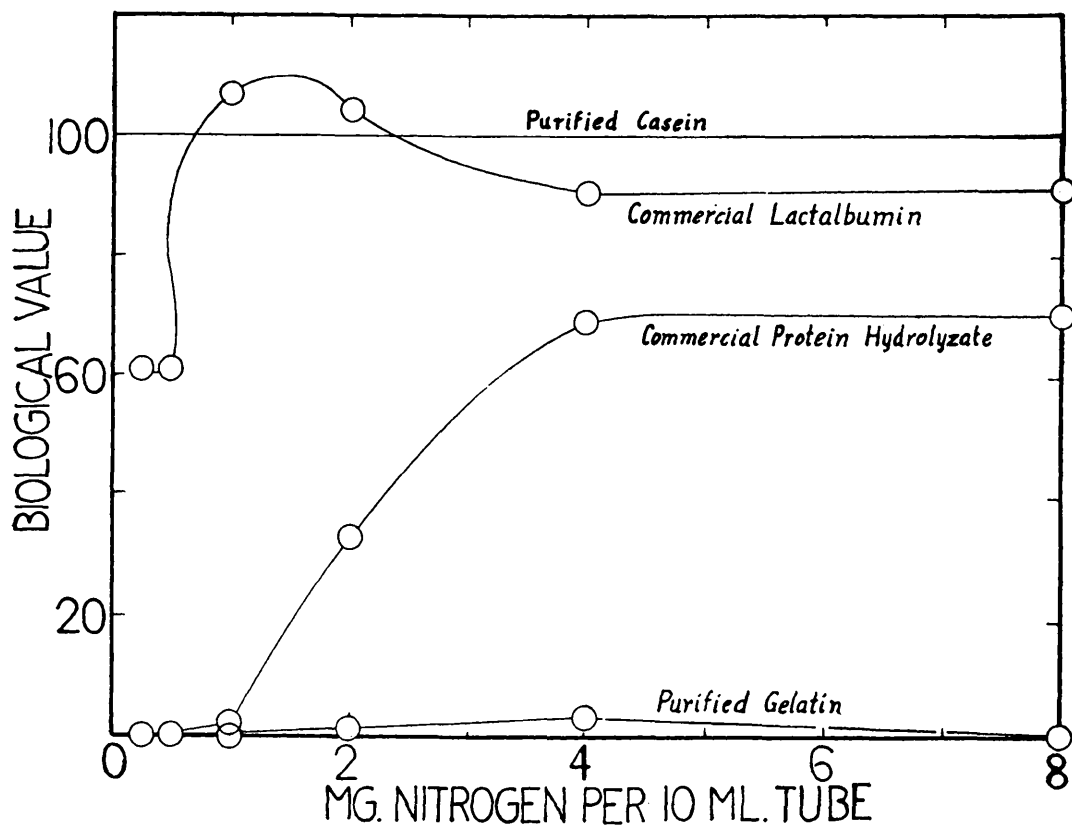


FIG. 1.
Curves relating biological value and nitrogen of proteins.

imate those of some mammals. It seems evident that biological values could be deter-

23, 601.

³ Harrison, H. C., and Long, C. N. H., *J. Biol. Chem.*, 1945, **161**, 545.

⁴ Mitchell, H. H., *Physiol. Rev.*, 1924, **4**, 424.

⁵ Madden, S. C., and Whipple, G. H., *Physiol. Rev.*, 1940, **20**, 194.

⁶ Barnes, R. H., Bates, M. J., and Maack, J. E., *J. Nutr.*, 1946, **32**, 535.

⁷ Cahill, W. M., *J. Am. Diet. Assn.*, 1945, **21**, 433.

⁸ Boas-Fixen, M. A., *Biochem. J.*, 1934, **28**, 592.

⁹ Boas-Fixen, M. A., *Nutr. Abstr. Rev.*, 1934-35, **4**, 447.

¹⁰ Allison, J. B., and Collaborators, *Annals New York Acad. Sci.*, 1946, **47**, 241.

¹¹ Block, R. J., and Mitchell, H. H., *Nutr. Abstr. Rev.*, 1946-1947, **16**, 249.

¹² Rockland, L. B., and Dunn, M. S., *Arch. Biochem.*, 1946, **11**, 541.

¹³ Kidder, G. W., and Dewey, V. C., *Physiol. Zool.*, 1945, **15**, 136.

mined with greater speed, convenience and economy with this organism than with larger animals and that errors due to biological variation would be minimized because of the large number of individuals and the aseptic conditions employed.

Experimental. A protein-free basal medium similar to that described by Kidder and Dewey,¹³ except for the omission of the amino acids, was employed in the present experiments. The technics were similar to those used in the authors' laboratory for the determination of amino acids with lactic acid bacteria. Aliquots of protein solutions, homogenized suspensions or hydrolyzates were added to 19 x 100 mm pyrex test tubes. Ten levels of sample, each in 2 or more tubes, were used. Protein- and amino acid-free medium and distilled water were added to a total volume of 10 ml per tube. The tubes were plugged, sterilized, inoculated with a

TABLE I.
Biological Values of Three Proteins.

Criterion	Animal	Protein			Reference No.
		Casein	Lactalbumin	Gelatin	
Liver wt regeneration	Rat	100	100	0	3
Nitrogen balance	Dog	100	137		14
Plasma protein regeneration	"	100	83		15
Protein level for optimum wt increase	Rat	100	133		16
Wt increase	Chick	100		46	17
" "	Rat	100	100-206*		16
Acid production	<i>T. geleii</i> H	100	61-112*	0-2*	This paper

* Range of values at different levels of sample.

washed suspension of *Tetrahymena geleii* H cells, and incubated at about 25° (room temperature) for varying lengths of time. The response of the organism was measured by electrometric titration with standard base.

Discussion. The biological values were calculated from the titration data using casein as the standard. The values found for lactalbumin, a protein hydrolyzate and gelatin varied with the incubation time and, as shown in Fig. 1, with the level of nitrogen in the sample. It has been shown previously by McCollum *et al.*¹⁸ and Plimmer *et al.*¹⁹ that biological values vary with the time and by Osborne *et al.*,¹⁶ Barnes *et al.*⁶ and other workers cited by these authors that they vary with the level of the protein.

¹⁴ Melnick, D., and Cowgill, G. R., *J. Nutrit.*, 1937, **13**, 401.

¹⁵ Melnick, D., Cowgill, G. R., and Buraek, E., *J. Exp. Med.*, 1936, **64**, 897.

¹⁶ Osborne, T. B., Mendel, L. B., and Ferry, E. L., *J. Biol. Chem.*, 1919, **37**, 223.

¹⁷ Almquist, H. J., Stokstad, E. L. R., and Halbrook, E. R., *J. Nutrit.*, 1935, **10**, 193.

¹⁸ McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, **23**, 231.

¹⁹ Plimmer, R. H. A., Rosedale, J. L., Raymond, W. H., and Lowndes, J., *Biochem. J.*, 1934, **28**, 1963.

The present data for 3 proteins, as well as the values reported in the literature, are summarized in Table I. The biological values found at some levels of samples by the authors' micro method are in approximate agreement with those obtained by other procedures. Although this correlation is of interest, it appears more significant that the true biological value of a protein is best expressed by a series of numbers if time, level of sample and possibly other variables are to be taken into account. It may be suggested that biological values of most meaning may be represented by areas under curves or volumes under planes. Whether or not there is a direct, or any, relation between the human requirements for proteins and the biological values of proteins determined with protozoa or other animals remains to be determined.

Further studies of this problem are in progress.

Summary. A method for the determination of biological values of proteins with *Tetrahymena geleii* H has been described. The values found for casein, lactalbumin, a commercial protein hydrolyzate and gelatin have been compared with each other and with those reported in the literature.