serum for the attachment of an incomplete antibody with negative results. Although these cells failed to show any evidence of sensitization after exposure to her serum *in vitro* at 37°C it was evident after repeated transfusions that these "compatible" cells shared in the rapid hemolysis *in vivo*. In other words, they were being destroyed by an immune body that could not be demonstrated in the patient's serum. It is probable that the immune body which caused the accelerated hemolysis in both patients was not demonstrable in the serum because it is adsorbed as it is produced by the large amount of cell antigen present.

It is not certain, however, that the particular body which was attached to the erythrocytes of both patients could not produce hemolysis or agglutination if present in sufficient concentration. Even if it is passive in this regard, the adherence of a protein to the cell surface probably exerts a detrimental effect by interfering with osmotic equilibrium. The spherocytosis observed may be produced by the accumulation of metabolites which raise the internal osmotic pressure and cause the absorption of fluid, thereby increasing osmotic and mechanical fragility of the erythrocyte and hastening its destruction. The failure to obtain agglutination of erythrocytes from patients with congenital hemolytic jaundice is compatible with the observations of Dacie and Mollison[11] that transfused cells have a normal longevity in this disease which suggests a cell defect rather than a hemolytic agent as the underlying cause.

Summary and Conclusions. The erythrocytes of 2 patients with acquired hemolytic anemia and spherocytosis have been shown to be readily agglutinated by an immune rabbit serum containing an antihuman globulin antibody. Since both patients have exhibited abnormal hemolytic activity for transfused cells this finding is compatible with the existence of an immune body type of hemolytic agent as the cause of the accelerated hemolysis.

Evidence has been presented to show that the immune substance can be separated from patients' cells by heating the cells to 56°C for 5 minutes and can then be combined with normal cells. The similarity of this immune body to the Rh blocking antibody is discussed. The demonstration of the presence or absence of an immune body attached to the erythrocytes should become a routine procedure in the study of hemolytic anemias.

11 Dacie, J. V., and Mollison, P. L., *Lancet*, 1943, **1**, 550.

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Role of Adrenalin in Recovery of Inhibited Conditioned Reactions.

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It was shown in earlier papers from this laboratory[1,2] that insulin coma and chemically- or electrically-induced convulsions cause the reappearance of previously inhibited conditioned reactions (c.r.). No distinct effect was exerted on positive c.r.'s by these procedures.[3,4] Since it had been shown previously[5,6] that the action which these proce-

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1 Gellhorn, E., and Minatoya, H. J., *J. Neurophysiol.*, 1943, **6**, 161.

2 Kessler, M., and Gellhorn, E., *Am. J. Psychiat.*, 1943, **99**, 687.

3 Gellhorn, E., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 155.

4 Gellhorn, E., *Arch. Neurol. and Psychiat.*, 1946, **56**, 216.

5 Gellhorn, E., *Arch. Neurol. and Psychiat.*, 1938, **40**, 125.

dures have in common is the excitation of the centers of the sympatheticoadrenal system, it was thought that the restoration of inhibited c.r.'s was likewise due to this increased reactivity of sympathetic centers. In order to clarify further the mechanism involved an attempt was made to determine whether the hypothalamic-cortical discharge, which occurs on excitation of diencephalic autonomic centers, (Murphy and Gellhorn)[7] or the release of adrenalin was responsible for the reestablishment of inhibited c.r.'s. For this purpose 2 series of experiments were performed. In the first, adrenalin was injected into normal animals whereas in the second group insulin coma was produced in adrenodemedullated animals. In both groups the effect of these procedures to restore c.r.'s which had been inhibited by lack of reinforcement was studied.

Methods. All experiments were performed on rats in which a definite escape reaction, as used in our earlier studies, had been established in response to the conditioned stimulus (bell or light). After the c.r. had been established it was inhibited by lack of reinforcement. Adrenalin (.2 mg per kg) was injected intraperitoneally 3 or 4 times on alternate days. The effect of these injections was compared with that induced by insulin coma or electroshock on 6 normal animals. The action of insulin coma was likewise studied in 6 adrenodemedullated rats. For further detail compare the literature cited above and the paper by Arnett and Gellhorn.[8]

Results and Discussion. Nineteen injections of adrenalin were performed on 6 normal rats in which preceding or following the adrenalin series the response of the rat to insulin coma or electroshock was studied. In all but one experiment no effect was elicited on the inhibited c.r.'s by the injection of adrenalin although the animals showed the typical recovery reaction under the influence of insulin coma or electroshock. In the one case in which the response of the c.r.'s was increased to 50% by adrenalin the effect was restricted to one day whereas a single injection of insulin leading to convulsion elicited in the same animal a marked recovery of the c.r.'s which lasted for 4 days and reached 90%. The experiments performed on adrenodemedullated rats led to a recovery (50% to 90%) of the c.r.'s after induction of an insulin coma. These experiments prove conclusively the fundamental difference between the effects of injected adrenalin and those of insulin coma and electroshock. Since in the latter conditions adrenalin is likewise involved, being secreted as the result of excitation of sympathetic centers, the difference, as far as restitution of inhibited c.r.'s is concerned, is based on a central action which occurs in insulin coma and electroshock and is not related to adrenalin.

The answer to this problem seems to lie in altered hypothalamic-cortical relations. Obrador,[9] Kennard,[10] and Murphy and Gellhorn[7] have demonstrated the profound effect which stimulation and destruction of the hypothalamus exert on electrical cortical activity. It may be assumed on this basis and on clinical evidence concerning autonomic relations in psychotic patients[6] that quantitative alterations in the activity of autonomic centers will in turn influence cortical functions.

It may be said that the reactivity of autonomic centers is greatly increased in insulin hypoglycemia[11] and in experimental convulsions.[12] This must lead to quantitative changes in cortical activity which may be the basis of alterations in behavior. Adrenalin, however, whether injected or secreted, far from causing a similar reaction, seems to depress autonomic centers and may be looked upon as a means of restoring increased ex-

6 Gellhorn, E., *Autonomic Regulations*, New York, 1943.

7 Murphy, J. P., and Gellhorn, E., *J. Neurophysiol.*, 1945, **8**, 341 and 431.

8 Arnett, V., Kessler, M., and Gellhorn, E., *Am. J. Physiol.*, 1942, **137**, 653.

9 Obrador, S., *J. Neurophysiol.*, 1942, **6**, 81.

10 Kennard, M. A., *J. Neurophysiol.*, 1943, **6**, 405.

11 Gellhorn, E., Ingraham, R. C., and Moldovsky, L., *J. Neurophysiol.*, 1938, **1**, 301.

12 Gellhorn, E., and Darrow, C. W., *Arch. Internat. Pharmacodyn.*, 1939, **62**, 114.

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

The statement by Himwich and Fazekas[14] 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 neurological 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 Tod[15] 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.

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

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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 protein[12] and that its amino acid requirements[13] 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,