No significant differences in hematocrit percentages were detected among corticosterone, deoxycorticosterone, and control groups. Such a lack of significant differences indicates that in this system the suppression by the steroids tested is not due to a hemolytic effect and is not associated with peripheral blood hemodilution following in the wake of sodium retention.

The time course for the erythropoietic depressor effect of deoxycorticosterone approximates that for the maximum acceleratory effect on erythropoiesis by testosterone[5]. In contrast, the abrupt corticosterone-induced depression of $^{59}$Fe incorporation is seen much earlier. If days are required for an alteration of peripheral red blood cell mass associated with changes in the production of erythropoietin, then it is conceivable that corticosterone affects the bone marrow directly, rather than the kidney or the liver's production of erythropoietic precursor substance. It is possible that erythroid cellular progression may be blocked at some stage(s), the total process may be 'turned-off', or the overall rate of progression may be reduced. The former possibility might not be the most favorable postulate, since it could be argued that if a cellular stage is blocked then there would be a damming-up of cells which are progenitors of the cells of the blocked stage; thus, when the depressor effect is alleviated one might observe an erythropoietic overshoot, i.e., a greater $^{59}$Fe incorporation percentage in relation to controls. Erythropoietic recovery from the effect of corticosterone is relatively gradual, and no overshoot is detected[6].

*Résumé.* Les stéroïdes surrénaux, déoxycorticostérone et corticostérone font diminuer l'érythropoïèse chez la souris femelle. La suppression produite par le corticostérone a lieu plus tôt et est plus grande que celle qui résulte du traitement au déoxycorticostérone.

G. R. HOGAN

*Department of Biology, University of North Carolina, P.O. Box 12665, Charlotte (North Carolina 28213, USA), 21 August 1970.*

[6] Supported by grants from the United Medical Research Foundation of North Carolina and the Foundation of the University of North Carolina at Charlotte.

## Enhancing Effect of *Bordetella pertussis* and Propranolol on Experimental Immune Hemolytic Anemia

Pertussis-vaccinated mice and rats display a heightened susceptibility to a wide variety of pharmacological, immunological, and physical stresses and stressor agents[1,2]. The $\beta$-adrenergic blocking agent, propranolol, shares with *Bordetella pertussis* the capacity to enhance sensitivity to several of these stressors[3–5], and it has been postulated that the sensitization effect of *B. pertussis* is primarily mediated through blockade of $\beta$-adrenergic receptors of the autonomic nervous system[6,7].

The observation, among others, that pertussis-inoculated mice have decreased blood sugar[7] and increased immunoreactive insulin levels[8] has led to the proposal of an inverse relationship between the glycemic state of a host and its susceptibility to a wide array of stressful stimuli[9,10]. In addition to their hypoglycemic effects, both *B. pertussis* and insulin are capable of inducing hypersensitivity to the pharmacological mediators, histamine and serotonin[7]. Both increase susceptibility to immediate[9], and delayed-type[11,12] hypersensitivity states, as well as to the reaction elicited by both carbohydrate[9,13,14] and non-carbohydrate[3,15] anaphylactoid agents. Additionally, we have found that insulin, like *B. pertussis*, can heighten the susceptibility of mice to bacterial endotoxins[16] as well as to the physical stress of hypoxic decompression[17]. Propranolol, which potentiates the hypoglycemic action of insulin[18,19], also has been shown to share some of these sensitizing properties[3–5].

Recently ADAMKIEWICZ et al.[20] reported that when mice are injected with rabbit antimouse erythrocyte serum, a complex hemolytic anemia syndrome develops. The symptoms include erythropenia, splenomegaly, hypoglycemia and death. These workers have demonstrated that the intensity of this syndrome is inversely proportional to blood sugar levels[20]. Hypoglycemia elicited either by fasting or by the injection of insulin rendered mice more suceptible to the lethal effect of antimouse erythrocyte serum[20]. Conversely, hyperglycemia induced by injection of either glucose or alloxan exerted a protective effect[20].

In view of the lengthening list of mouse-sensitizing properties shared by insulin, *B. pertussis*, and propranolol, we considered it of interest to determine whether the latter two agents could, like insulin, enhance the

[1] L. LEVINE and R. E. PIERONI, Experientia *22*, 797 (1966).
[2] J. MUNOZ and R. K. BERGMAN, Bact. Rev. *32*, 103 (1968).
[3] R. E. PIERONI and L. LEVINE, J. Allergy *39*, 25 (1967).
[4] R. G. TOWNLEY, I. L. TRAPANI and A. SZENTIVANYI, J. Allergy *39*, 177 (1967).
[5] S. MALKIEL and B. J. HARGIS, Proc. Soc. exp. Biol. Med. *125*, 565 (1967).
[6] R. E. PIERONI and L. LEVINE, Nature *213*, 1015 (1967).
[7] C. W. FISHEL, A. SZENTIVANYI and D. W. TALMAGE, in *Bacterial Endotoxins* (Ed. M. LANDY and W. BRAUN; Rutgers University Press, New Jersey 1964), p. 474.
[8] A. GULBENKIAN, L. SCHOBERT, C. NIXON and I. I. TABACHNICK, Endocrinology *83*, 885 (1968).
[9] V. W. ADAMKIEWICZ, Can. med. Ass. J. *88*, 806 (1963).
[10] R. E. PIERONI and L. LEVINE, Fedn Proc. *26*, 802 (1967).
[11] G. E. THOMPSON, Nature *215*, 748 (1967).
[12] D. A. ROWLEY, J. CHUTKOW and C. ATTIG, J. exp. Med. *110*, 751 (1959).
[13] A. GOTH, W. L. NASH, M. NAGLER and J. HOLMAN, Am. J. Physiol. *191*, 25 (1957).
[14] S. MALKIEL and B. J. HARGIS, Int. Arch. Allergy *34*, 75 (1968).
[15] R. E. PIERONI and L. LEVINE, Experientia *25*, 170 (1969).
[16] R. E. PIERONI and L. LEVINE, Experientia *25*, 507 (1969).
[17] R. E. PIERONI, E. J. BRODERICK and L. LEVINE, Experientia *27*, 164 (1971).
[18] E. A. ABRAMSON, R. A. ARKY and K. A. WOEBER, Lancet *2*, 1386 (1966).
[19] M. N. KOTLER, L. BERMAN and A. H. RUBENSTEIN, Lancet *2*, 1389 (1966).
[20] V. W. ADAMKIEWICZ, V. H. SKANES and P. J. SACRA, Can. J. Physiol. Pharmac. *46*, 621 (1968).

sensitivity of mice to experimental immune hemolytic anemia.

*Materials and methods.* The rabbit antimouse erythrocyte serum used in these studies was prepared and generously supplied to us by Dr. V. ADAMKIEWICZ. Its method of preparation and properties have been previously described[20]. The pooled antiserum had an agglutinating titer of 1024 agglutinin units per ml, as measured against fresh mouse erythrocytes[20]. The histamine-sensitizing factor (HSF) of *B. pertussis* was prepared as previously described[21]. This proteinaceous component of the bacillus is believed responsible, not only for the enhanced sensitivity to histamine observed in pertussis-inoculated mice, but also for other sensitizing effects of *B. pertussis*[1-3]. Propranolol (Inderal, Ayerst Labs) was generously supplied by Dr. SAHAGIAN-EDWARDS. CFW female mice, weighing between 14 and 20 g were obtained from Carworth Farms, New City, New York. 3 groups of 20 mice received i.p. injections of saline, 45 µg *N* of HSF (4 days before challenge), or 1 mg of propranolol (10 min before challenge). 2 additional control groups of 20 mice received HSF and propranolol only. The first 3 groups were then injected i.p. with 25 agglutinin units of antiserum. All 5 groups were then observed for 72 h for signs of toxicity and mortality, the latter being used as the end-point in determining any enhanced susceptibility to the experimental hemolytic state, as described by ADAMKIEWICZ et al.[20].

Effect of histamine-sensitizing factor (HSF) of *B. pertussis*, and propranolol on susceptibility of CFW mice to experimental immune hemolytic anemia

| Sensitizing agent | Dose | Rabbit antimouse erythrocyte serum (agglutinin units) | Death rate |
|---|---|---|---|
| Saline | 0.5 ml | 25 | 1/20 |
| HSF | 45 µg N | 25 | 18/20 |
| Propranolol | 1 mg | 25 | 14/20 |
| HSF | 45 µg N | – | 1/20 |
| Propranolol | 1 mg | – | 0/20 |

All injections i.p. HSF injected 4 days prior to antiserum. Propranolol injected 10 min before antiserum. Deaths tabulated 72 h after antiserum challenge. Death rates from HSF plus antiserum: Chi-square for separate effects versus combined effect 43.3: $P < 0.001$. Death rates from propranolol plus antiserum: Chi-square for separate effects versus combined effect 32.4: $P < 0.001$.

*Results and discussion.* Very shortly after injection of the antiserum it was observed that the HSF- and propranolol-inoculated animals exhibited severe toxic symptoms. These symptoms included ruffling of the fur, tachypnea, prostration, and occasional convulsions. These signs were absent, or present to only a minor degree, in saline-injected controls receiving antiserum. The Table shows that 72 h after antiserum challenge the groups that had received antiserum alone, HSF alone or propranolol alone had death rates of only 1/20, 1/20, and 0/20, respectively. On the other hand, the groups that received HSF and antiserum or propranolol and antiserum had death rates of 18/20 and 14/20 respectively ($P < 0.001$). Thus there was clear potentiation of the lethal effect of the antiserum by both HSF and propranolol. A second experiment of like design produced similar results.

These results show that a component of *B. pertussis*, as well as the β-adrenergic blocking drug, propranolol, as had earlier been reported with insulin, are capable of enhancing the susceptibility of mice to experimental immune hemolytic anemia. Studies are currently underway to explore the mechanism(s) involved in the enhancing effect of the 3 agents on this hemolytic state, and to investigate further the role of blood and tissue glucose levels in the susceptibility of experimental animals to stressful stimuli[22].

*Résumé.* Un constituant de *Bordetella pertussis*, et la drogue antagoniste, le propranolol β-adrénergique, peuvent causer un renforcement semblable similaire de la sensibilité des souris à l'anémie hémolytique immune expérimentale. Il est connu que l'insuline se comporte de la même façon.

R. PIERONI, AMINA E. BUNDEALLY and L. LEVINE

*Division of Biologic Laboratories, State Laboratory Institute, Massachusetts Department of Public Health, and the Department of Microbiology, Harvard School of Public Health, Boston (Massachusetts 02130, USA), 12 February 1970.*

[21] R. E. PIERONI, E. J. BRODERICK and L. LEVINE, J. Immun. *95*, 643 (1965).

[22] This work was supported in part by Public Health Service Research Grant No. CC00223 from the National Communicable Disease Center through the Massachusetts Health Research Institute.

## A Probable Effect of Diabetes Mellitus on Incorporation of Amino Acids into Proteins Catalyzed by Isolated Rat Liver Nuclei

Whether hormones alter nuclear protein synthesis, believed to occur in vivo, is not certain, due to the flux of proteins across intracellular compartments[1-3]. The effect of alloxan-induced diabetes mellitus on the ability of isolated rat liver nuclei to catalyze incorporation of radioactive amino acids into hot TCA-insoluble materials (labeling of nuclear proteins) has been examined. Preparations from diabetic rats often exhibited reduced incorporation, which insulin in vivo could restore toward normal.

*Methods.* Male Sprague Dawley or Long Evans rats, rendered diabetic by i.v. injection of 50 mg of alloxan in 0.9% saline/kg body weight, were maintained on

[1] V. G. ALLFREY, in *Protein Biosynthesis* (Ed. C. B. ANFINSEN; Academic Press, New York 1970), p. 247.

[2] L. GOLDSTEIN and D. M. PRESCOTT, J. Cell Biol. *33*, 637 (1967).

[3] E. ROBBINS and T. W. BORUN, Proc. natn. Acad. Sci., USA *57*, 409 (1967).