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(NASA-CR-163591) CHANGES IN ERYTHROPOIETIN LEVELS DURING SPACE FLIGHT OR SPACE FLIGHT SIMULATION Final Technical Report **(Tennessee Univ.)** 8 p HC A02/MF A01 CSCL 06P G3/52

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The final Technical Report on contract NAS 9-15164 attempts to summarize the major achievements of the work carried out since October 1976. It is by way of review of the data - detailed results can be found in our quarterly reports and in the progress reports submitted as part of our contract renewals.

1. Space Flight Simulation Studies: Two hundred and seventy samples from 24 subjects involved in 3 bedrest studies and from 3 subjects involved in Spacelab Mission Development Test III were assayed for erythropoietin (Ep), in an *in vitro* fetal mouse liver cell assay, and for ferritin using a commercially-available immunoradiometric assay kit. Despite a small but significant decrement in red cell mass in all subjects no trends or significant changes in serum Ep were observed. On the other hand, serum ferritin concentrations tended to increase slightly during the "missions" perhaps reflecting a redirection of iron from the suppressed erythron into iron stores.

2. Evaluation of Inflight Blood Collection Tubes (IBCT): A considerable amount of effort was directed at determining the suitability of the proposed IBCT to provide serum for Ep and ferritin assays. Given the limited number of flight tubes available, uncertainty as to the exact temperature profile to which the samples will be exposed during flight and the mechanical dysfunction of many of the tubes supplied to us, any conclusions must be tentative. The data we have been able to collect do not suggest any problems will be encountered with the proposed IBCT in so far as providing material for Ep and ferritin assays are concerned.

3. Development of a method for hemoglobin p50 determinations on stored blood: Shifts in the hemoglobin oxygen dissociation curve (characterized by the p50) have been theorized to be involved in the "anemia" of space flight.

An investigation of hemoglobin p50 shifts during space flight is problematical because of the lability of this component. We have demonstrated that the p50 in blood stored at +4°C in heparinized micro-hematocrit tubes is stable for at least 7 days. Therefore, on the basis of our results and providing the storage conditions can be satisfied (and the temperature and storage container are quite critical) an evaluation of hemoglobin p50 shifts during space flight would seem feasible.

4. Documentation: In observance of our contractural requirements the following two documents were submitted to the Technical Monitor in May 1979;

a. Hormonal Control of Erythropoiesis

b. A Comparison of Erythropoietin Assays with Particular Emphasis on their Suitability for Investigations into the "Anemia" of Spaceflight.

5. Animal and Computer Studies into the "Anemia" of Space flight:

a. Dehydrated Mice: We have shown that mice deprived of water have in common with men in space hemoconcentration, secondary to a plasma volume reduction, and a negative energy balance. Many studies, supported by a mathematical model for the regulation of erythropoiesis were undertaken to elucidate the relative importance of these two parameters in producing the resulting anemia. These investigations suggested that, in dehydrated mice, the major cause of the anemia (expressed as an absolute volume of red cells) was the negative energy balance with hemoconcentration playing a minor role. Perhaps the anemia represents an "adaptation" to a smaller body weight as the red cell mass, when expressed as ml/gm body weight, was essentially normal.

Despite the superficial similarities of dehydrated mice and men in space, there is a considerable "creditability gap" between a four-legged,

20 gm animal in 1g and a two-legged, 70 kg animal in 0 g. To, at least partially, overcome this gap experiments in small primates were undertaken:

b. Dehydrated marmosets: Studies in dehydrated marmosets were undertaken preparatory to work on squirrel monkeys. Complete water deprivation of marmosets resulted in erythropoietic changes quite different from those observed in mice under a similar experimental protocol. The limited amount of data suggested that if dehydration affected erythropoiesis at all in marmosets it was to stimulate, nor inhibit, red cell production. Of revelance to space flight was a shift in the hemoglobin p50 of marmoset blood during dehydration.

c. Squirrel monkeys: Under another NASA contract, Dr. M. Moore-Ede at Harvard Medical School has shown that squirrel monkeys exposed to lower body positive pressure (LBPP) exhibit endocrine and electrolyte changes similar to those seen in men during space flight. Towards the end of our contract period, a small colony of squirrel monkeys was established to investigate the effects of LBPP on erythropoiesis. In this facet of our work, the time was spent obtaining the necessary animals and equipment and training and catheterizing the monkeys. Erythroproietic research will commence after the monkeys, necessary equipment and NASA funds have been relocated at the Baylor College of Medicine.

6. Modifications of Ep assays: Under this contract our routine *in vivo* and *in vitro* assays were modified and/or simplified to take into account recent methodological advances. Two studies, partially supported by NASA, demonstrated a correlation between Ep titers detected with an *in vitro* fetal mouse liver cell assay and those detected in a standard, *in vivo*, ex-hypoxic mouse system.

7. Development of new Ep assays: All current assays for Ep require whole animals, animal tissue and/or sophisticated radiation counting devices. As such they are probably not suitable for use in a space flight environment. Under the aegis of this contract considerable progress was made towards the development of an Ep assay for potential inflight use. This system depends on the ability of Ep - containing materials to produce "rockets" when subjected to electrophoresis in an agar gel containing wheat germ lectin (WGL). The potential use of such an assay was identified but problems of specificity due to impure Ep and WGL prevented a definitive resolution. Similar problems were encountered in the development of a, unique, two-site immunoradiometric assay using WGL and Ep-specific, radio-iodinated antibodies.

8. Publications:

a. Dunn, C. D. R., Preston, J., and Lange, R. D. Serum erythropoietin titers during bedrest. In Final Report. Johnson Space Center - Methodist Hospital 28-Day Bedrest Simulation of Skylab. Volume II. Johnson, P. C. and Mitchell, C. (Eds.). Lyndon B. Johnson Space Center, pp. D60-D-83, 1977.

b. Dunn, C. D. R. The effect of dehydration on erythropoiesis in mice: Relevance to the "anemia" of space flight. Aviat. Space and Environ. Med., 49:990-993, 1978.

c. Kimzey, S. L., Johnson, P. C., and Dunn, C. D. R. The influence of spaceflight on erythrokinetics in man: SMD III, Experiment 68. In Final Report. Spacelab Mission Development Test III, Volume I Scientific Experiments. Lyndon B. Johnson Space Center, JSC-13950, pp. 309-345, 1978.

d. Dunn, C. D. R., and Lange, R. D. Erythroid regulatory factors. Correlation of *in vivo* and *in vitro* detectable titers. Blood, 52:1238-1242, 1978.

e. Dunn, C. D. R., and Lange, R. D. Erythropoietic effects of spaceflight: Further studies in a potential animal model. Acta Astronautica, 6:725-732, 1979.

f. Dunn, C. D. R., and Do, N. The stability of erythroid stimulating activity in normal human serum. Biochem. Med., 21:190-195, 1979.

g. Dunn, C. D. R., Lange, R. D., and Jones, J. B. Evidence that the fetal mouse liver cell assay detects Erythroid Regulatory Factors (ERF) not determined with a standard in vivo assay. Exp. Hemat., 7:519-523, 1979.

h. Dunn, C. D. R. The effect of food and water restriction on erythropoiesis in mice: Relevance to the anemia of space flight. Amer. J. Physiol., 238:R301-R305, 1980.

i. Dunn, C. D. R., and Lange, R. D. Erythropoietin: Assay and Characterization. In: "Topical Reviews in Hematology". Roath, S. (Ed.). John Wright and Sons Ltd., Bristol. In Press.

j. Dunn, C. D. R., and Smith, L. N. The effect of dehydration on erythroid progenitor cells in mice. Exp. Hematol., 8:620-625, 1980.

k. Dunn, C. D. R., and Lange, R. D. Erythropoietin titers in normal human serum. An appraisal of assay techniques. Exp. Hemat., 8: 231-235, 1980.

l. Leonard, J. I., Kimzey, S. L., and Dunn, C. D. R. Dynamic regulation of erythropoiesis: A computer model of general applicability. Exp. Hemat., In Press.

m. Dunn, C. D. R., Smith, L. N., Leonard, J. I., Andrews, R. B., and Lange, R. D. Animal and computer investigations into the murine response to chronic hypoxia. Exp. Hemat., In Press.

n. Dunn, C. D. R., and Trent, D. The effect of parathyroid hormone on erythropoiesis in serum-free cultures of fetal mouse liver cells. Submitted.

o. Dunn, C. D. R., Leonard, J. I., and Kimzey, S. L. Interactions of animal and computer models in investigations of the "anemia" of space flight. In preparation.

9. Presentations at Meetings:

a. Dunn, C. D. R., Preston, J. P., Lange, R. D., and Kimzey, S. L. Serum erythropoietin levels during bedrest. Presented at the 48th Annual Scientific Meeting of the Aerospace Medical Association. Las Vegas, 1977.

b. Dunn, C. D. R. Erythropoiesis in mice with absolute and relative increases in red cell mass (RCM). Proceedings of the 7th Annual Meeting of the International Society for Experimental Hematology. Chicago. Exp. Hemat., 6 (Suppl. 3):57, 1978 (Abstract).

c. Dunn, C. D. R., and Lange, R. D. The "anemia" of space flight. Some considerations from a potential animal model. Proceedings of the 21st Annual Meeting of the American Society of Hematology. New Orleans, La. Blood, 52 (Suppl. 1):384, 1978 (Abstract).

d. Dunn, C. D. R., and Lange, R. D. Erythropoietic effects of space flight studied in a potential animal model. Proceedings of the 50th Annual Scientific Meeting of the Aerospace Medical Association, Washington, D.C., pp. 14-15, 1979 (Preprint).

e. Leonard, J. I., Dunn, C. D. R., and Kimzey, S. L. Computer simulation of erythropoietic suppression in a potential animal model for space flight. Proceedings of the 50th Annual Scientific Meeting of the Aerospace Medical Association, Washington, D.C., pp. 16-17, 1979 (Preprint).

f. Dunn, C. D. R., and Lange, R. D. The erythropoietic effects of space flight studied in a potential animal model. Proceedings of the 8th Annual Meeting of the International Society for Experimental Hematology, Rotterdam, Exp. Hemat. 7 (Suppl. 6):37, 1979 (Abstract).

g. Smith, L. N., and Dunn, C. D. R. The effect of dehydration on erythroid progenitor cells. The Proceedings of the Annual Scientific Meeting of the Southeastern Section of the Society for Experimental Biology and Medicine, Oak Ridge, Volume 4, Abstract #14-S (1979).

h. Dunn, C. D. R., and Trent, D. Modulation of erythropoiesis by parathyroid hormone (PTH) in serum-free cultures of fetal mouse liver cells. Proceedings of the 9th Annual Meeting of the International Society for Experimental Hematology, Dallas, Ext. Hemat., 8 (Suppl. 7):126, 1980 (Abstract).