BIOCHEMICAL ASPECTS OF ACCLIMATIZATION OF MAN TO HIGH ALTITUDE STRESS

K. K. Srivastava

Defence Institute of Physiology & Allied Sciences, Delhi Cantt

(*Received* 6 *August* 1974)

The paper reviews the biochemical aspects of acclimatization of human body to high altitude with particular reference to the adaptive changes in skeletal muscles, hepatic function, adrenal function and carbohydrate metabolism.

Exposure at high terrestrial altitudes constitutes a general systemic stress. Decrease in partial pressure of atmospheric oxygen is the dominating factor that causes this stress at altitude. As a result of this stress the cardio-respiratory system in man starts rapid functioning in order to meet the normal oxygen requirement of the tissues. In case, the oxygen supply falls short of the optimum requirement of the tissues, the latter invoke adaptive mechanisms for survival and function at low oxygen tensions. The low atmospheric pressure in tissue exerts a stress of different sort[1]. Severe cold conditions prevailing at high altitudes, further aggravate the oxygen deficiency in view of its increased demand for thermogenesis. Besides, several systemic responses to hypoxia are diametrically opposite to those at low ambient temperature and hence complicate the physiological responses to natural high altitude conditions[2]. The ultraviolet and ionizing radiations at altitudes may also complicate the systemic response. A localized variation in ozone content of the atmosphere at high altitudes may further add to the respiratory distress[3]. These multiple factors strongly suggest that studies on the effects of high altitude should not be conducted under simulated conditions of hypoxia, cold and radiation separately. Such studies though of immense interest, are often unable to predict the course of events at high terrestrial altitudes.

Most of the studies on the effect of high altitude and acclimatization have been restricted to few weeks. The effect of high altitude during prolonged stay has been evaluated from the data obtained on native populations. Since the natives of high altitude area represent a maximally adapted population to high altitudes, the mechanisms and course of adaptation in persons residing at plains can not be predicted from these studies. An opportunity for such studies was, however, provided by the Indian army personnel who were stationed at altitudes beyond 10,000 feet for prolonged periods of two to three years. The army population further facilitated the control and selection of many variables otherwise extremely difficult to control in natives, such as age and physique, dietary variety, caloric and nutritional intake, physical conditioning and work output. For biochemical evaluation, these controls are necessary. As a result, considerable research activity emanated in the field of high altitude physiology and biochemistry in India particularly in Defence Laboratories. In this article, biochemical aspects of this study and some other connected aspects of high altitude biochemistry have been discussed. A large field of research in clinical medicine, treatment and pathology of high altitude diseases wherein considerable contribution of Indian Defence Scientists exists have been left out.

PHYSIOLOGICAL ADAPTATIONS CONCERNING OXYGEN TRANSPORT

A large decrease in ambient partial pressure of oxygen at high altitude is associated with a comparatively small decrease in venous oxygen tension in man[4]. Evidently the cardio-respiratory system undergoes profound alteration in its function to meet the challenge of oxygen deficiency. The increase in ventilation or respiratory rate appears to be the primary response in this direction[5]. In lungs, arterial oxygenation of the blood is facilitated by increased diffusion capacity; the latter has been observed in natives of high altitude[6] area but could not be confirmed in acclimatized lowlanders[7]. Among the latter, the oxygen carrying capacity of the blood is increased which manifests in the form of increased hemoglobin and hematocrit. The oxy-hemoglobin dissociation curve shifts to the right[8]. This presumably facilitates the delivery of oxygen to the tissues at reduced oxygen tension. The role of diphosphoglycerate in shift of the oxygen hemoglobin dissociation has been recognised[9]. However, the significance of such a shift with reference to high altitude adaptation in man has been questioned[10]. Besides, a number of animal species which are fully acclimatized to high altitudes do not possess diphosphoglycerate in their red blood corpuscles[11].

Prolonged exposure to hypoxia and hypoxic environment brings about extensive capillarization of tissues[12]. This also helps in reducing the oxygen gradient from circulating blood to cellular structures. An increase in the myoglobin content of the tissues[13] appears to facilitate the diffusion of oxygen and its storage in tissues.

The tissues at certain critical altitude or hypoxia are unable to receive sufficient oxygen for their optimal functioning, inspite of the enhanced capacity for oxygen intake, increased oxygen capacity of the vasculature and increased diffusion in the cellular structures[14]. Under these conditions, the anaerobic metabolism is forced to operate. If the oxygen deficiency is further prolonged or increased, changes in cellular mechanisms develop as adaptive process to utilize the available oxygen most economically. In well adapted high altitude populations, critical oxygen pressures for several processes such as maintenance of rectal temperatures, cardiac output, oxygen consumption, are far lower than in sea level populations[14]. Such observations strongly suggest that not only the body mechanisms adapt to high altitude by increasing their efficiency for oxygen supply but also endeavour to utilize the available oxygen most economically.

ADAPTATIONS IN TISSUES

The acclimatization to prolonged hypoxia or terrestrial altitudes requires several adaptive changes in tissues at cellular and sub-cellular level. Different tissues follow a differential time schedule for adaptations to ensue because of the different amounts of oxygen required, (*ii*) differential blood flow pattern or oxygen supply and the effect of hygoxia on such blood flow patterns, (*iii*) inherent capacity of the tissue to change in response to hypoxia. Hence, it is desirable to discuss the acclimatization phenomenon of human body to high altitude hypoxia at cellular level, tissue wise.

ADAPTIVE CHANGES IN SKELETAL MUSCLES

More than half of the body weight is contributed by skeletal muscles. Skeletal muscles are also concerned with physical movement and energy expenditure. Hence, the effect of oxygen deficiency on the performance and functioning of skeletal muscles is expected to be of a very large magnitude. Physical work performance and its efficiency in terms of energy expenditure has been found to be considerably decreased among the persons who come from plains to the high altitude[15]. It takes up a considerable period of acclimatization for attaining a reasonable efficiency in work performance at altitude [16,17]. The reason for the loss of efficiency at altitude has been widely believed to be the limitation of cardio-respiratory system to supply adequate oxygen to working muscles. During such work, the energy cost of the function is also increased due to additional energy requirement of respiratory muscles[18] which have to work at a higher pace.

The cardio-respiratory system undergoes considerable adaptive changes. There are changes in functioning of lungs[19] cardiac muscle[20] vascularization of tissues etc. But all these are not sufficient to provide adequate oxygenation as the anaerebic load on the working muscle increases progressively at altitude[21,22,23]. In adapted individuals, more fundamental changes indicative of metabolic modifications are seen. The lactate accumulation in response to maximum exercise is less among acclimatized individuals as compared with the unadapted persons from the plains and higher the altitude of acclimatization, the lower is the lactate accumulation[21,24,25]. Recently, an increase in succinate accumulation was observed[26] in experimental animals in response to chronichypoxia. It has been suggested that lactate is further transformed into succinate in high altitude adapted animals. The relationship of such a finding in animals is yet to be explored in men. The decreased accumulation of lactate at altitude might also be due to increased alkalinity of the blood or reduced muscular glycogen content[27].

Inspite of the limitation on the performance of physical work, the man, specially the soldier, has to perform strenous physical tasks at high altitude. Whether such a physical endeavour would lead to any abnormality in musculature was an open question, in view of the muscle wasting[28] negative nitrogen balance and appetite loss[29] which have been often observed at moderate to high altitudes. During the course of two years of stay at an altitude of nearly 4000 metres, measurements of serum creatine phosphokinase levels (SCPK) and simultaneous excretion of creatine and creatinine were made[30]. The enzyme level in circulation increased up to tenth month of stay at altitude followed with a progressive decrease with the duration of stay. An increase in circulating creatine phosphokinase would normally suggest either a muscular or neural pathology. However, muscular dystrophy or necrosis is generally accompanied with increased urinary excretion of creatine and creatinine coefficient. The former was elevated only during first two weeks of altitude stay and the latter remained more or less constant for two years at altitude. The rise in SCPK, therefore, could not be due to overt muscular damage. There was no significant rise in serum aminotransferase levels at altitude except for during first two weeks[31], an observation, which further confirms that there was no cardiac or skeletal muscle damage at altitude in these men. Further, evidence in this connection was available from studies on the efficiency of physical work performance which showed progressive improvement with the duration of stay. During tenth month of altitude exposure, the loss in body weight of these soldiers was barely 500 gms and in fact, their lean body mass was higher than the initial sea level value[32]. These observations suggest that there was no damage to musculature which could be responsible for the increase in serum creatine phosphokinase levels.

The urinary excretion of creatinine was generally increased at altitude among lowlanders. The natives had also a higher turnover of creatinine at altitude[36]. These observations might be indicative of higher rate of metabolism in muscles. An increased myoglobin content[13] and enzyme activities [33] including the mitochondrial mass[34] have been reported in muscles of high altitude animals and man. It is obvious, that the decreased availability of oxygen poses sufficient threat to the survival of body musculature at altitude but the tissue is able to adapt successfully at least at moderate altitudes.

HEPATIC FUNCTION AT ALTITUDE

Liver has a great capacity to survive with low oxygen supply. Yet, it has to perform a variety of inter-organ functions in the body and hence, the abnormality in other organs, affects the hepatic function. Thus, the lactate accumulation in skeletal muscle has to be compensated by its increased utilization or oxidation in the liver.

In our studies, profound changes in serum proteins were observed at altitude[35,36]. During first two weeks of exposure, an increase in total serum protein concentration was observed. This is widely believed to be due to hemoconcentration. However, this was accompanied with a decrease in albumin to globulin ratio. Subsequently the protein concentration returned to normal level in serum but the change in albumin to globulin ratio persisted even up to four weeks after return to sea level from altitude. There was an absolute decrease in albumin and increase in globulin fractions. The most prominent increase in globulins was seen in γ-fraction up to 20th month of stay this subsequently changed over to β and α_2 fractions. The α_2 and β globulins were significantly higher in natives of high altitude area as compared with lowlander[36]. The increase in γ-globulins among lowlanders at altitude was not accompanied with rise in serum aminotransferase activities[31]. The rise in globulins specially in γ-fraction would clinically suggest a hepatic disorder or infective state. Since, the aminotransferase levels were maintained around sea level value, this could not be the case. The dye and galactose elimination studies also suggest a normal hepatic function during first week of altitude exposure, when the hypoxic effect of environment is supposed to be maximum in man[37]. The changes in albumin and globulin are presumably adaptive process. The reasons for the increase in globulins at altitude can only be surmised. It could be due to a rapid turnover of lymphocytes or reticuloendothdial system specially in lungs. The increased α_2 and β fractions may help in transporting materials required for rapid erythropoesis and hyperactive adrenals. The decrease in serum albumin might be due either to a partial inhibition in its synthesis[38] or its transfer from intravascular bed to extravascular space[39].

The fatty infiltration of liver was reported as the cause of heavy mortality of neonatal rats at high altitude by Chiodi[40] et al. Possibly, the fatty infiltration of liver does not occur in adult man or in adult animal at altitude. The hyperfunction of adrenals during early high altitude exposure generally results in lipolytic mobilization of fats from adipose tissue and has been considered to be the primary cause of loss of body weight[41]. Under these conditions, an increase in circulating free fatty acids has been observed and with the passage of time and with successful acclimatization, the circulating free fatty acids return to normal level[42]. A shift in respiratory quotient also suggests enhanced oxidation of fats over carbohydrates during early high altitude exposure[5, 43].

There was a distinct decrease in circulating cholesterol in man at altitude[36]. This happens inspite of the increased fat and caloric intake. Man at moderately high altitude can digest increased intake of fats as well[44]. This efficient digestibility of fats without increase in circulating fats and fatty acids suggest an efficient removal and utilization of fats by liver at altitude, indicating a normal, hepatic functioning at altitude at least up to 4000 metres.

RENAL FUNCTION AT ALTITUDE

Lowlanders exposed to an altitude beyond 3500 metres and natives of high altitude area excrete larger amount of urinary protein and show reduced creatinine clearances[45-47]. This effect of high altitude on glomerular filtration has been ascribed to hypoxia induced interference in protein reabsorption and partly to altered renal blood flow.

However, in our studies urinary excretion of protein at or near 3500 metres in normal man during first week of exposure varied irrespective of climatic changes, physical exertion of the individual and day of exposure to altitude. On the other hand in men, who had suffered from high altitude pulmonary oedema on a previous sojourn, an increased urinary output of protein was observed on first day of altitude exposure. Subsequently the protein excretion decreased but again had no correlation with climatic variation, physical exertion or days of altitude exposure.

The studies on urea clearance of man during prolonged stay at altitude indicate an interference in renal filtration of urea in lowlanders[36]. At the same time, natives had a normal urea clearance[36]. It is not clear whether the interference in urea clearance of lowlanders at altitude is of any significance. For changes to occur in blood urea levels, a very large decrease in urea clearance is required. It is not surprising therefore that men at high altitude did not feel any discomfort or abnormality. The interference in urea clearance was also only of temporary nature as on return to sea level, normal clearance was observed within first week.

ADRENAL FUNCTION AT HIGH ALTITUDE

Several workers have observed hyper function of adrenal glands on acute exposure to high altitude. Generally, the adrenals quickly adapt to new environment and return to normalcy, within first week of high altitude stay[48-50]. However, Halhuber and Gable[51] reported a wide fluctuation in urinary excretion of steroids during third week of exposure. Klein[52] found sixty percent higher corticoid level even after 6 weeks of altitude stay. Evidence for increased adrenal activity has been obtained in experimental animals for 3-4 months of continuous simulated altitude exposure[53]. On the other hand, no significant increase in adrenal activity was observed among mountaineers even though they faced extremes of environmental conditions[54]. An increased 11-hydroxy corticoid in blood was observed in young mountaineers only on days of considerable physical exertion[55]. Pugh[56] had earlier suggested an altitude induced depression in gonadal and androgenic functions based on his observations on an Everest expedition. MacInnes et al[55] have observed a decrease in 11-Hydroxy corticoid levels in blood in exhausted mountaineers. It appears from these observations that several regulatory factors such as extent of altitude and associated hypoxia, variation in environmental temperature, physical status of the individual and physical exertion are involved in acclimatization of adrenal glands and that the adrenals remain responsive to other factors even after acclimatization to hypoxia. Hence a seasonal variation, similar to experimental animals, may also be observed in man. In man during two years of stay at altitude, increase in 17-Keto-steroid excretion appeared to occur with the lowering of ambient temperature. However, the 17-hydroxy corticoid increased only during first 10 months of stay. Subsequently, it decreased to sea level value and did not change with seasons. The mechanism of differential response in 17-Keto and 17-Hydroxy steroid excretion due to a change in environmental conditions is not clear. In case, the ACTH stimulates the retention of cortisol in tissues with acclimatization, the urinary loss of cortisol metabolites (17-OHCs) may not be observed with the increase in 17-Ketosteroids excretion. The natives of high altitude area had a significantly higher urinary excretion of 17-Ketosteroids and 17 hydroxy steroids compared with lowlanders at sea level and after acclimatization to high altitude[36]. Moncloa and Prettel[57] on the other hand, found similar urinary output of steroids among Peruvian natives and sea level residents. The reasons for the difference between Ladakhis and Peruvian natives are not clear.

STATUS OF CARBOHYDRATE METABOLISM AT HIGH ALTITUDE

A depressed utilization of glucose[58-60] has been reported by several investigators at high altitude. A depressed oxidation of glucose and its conversion to fatty acids were also observed in high altitude exposed rats[59]. In contrast to these findings, high carbohydrate diets have been found to be beneficial and palatable by many investigators at high altitude. It appears that the homeostasis of glucose between its production and intake on the one hand and oxidation and its conversion to other metabolites, on the other, are affected by more than one factors at high altitude. Duration of stay was found to be the factor affecting blood glucose levels in our investigations[36,64]. The blood sugar level in fasting men increased up to tenth month of stay at altitude and then it progressively decreased even below the normal sea level value at the end of 24 months of stay. However, during two years of stay, the glucose utilization as indicated by the tolerance studies was not affected. This suggests that the insulin response to additional glucose load was operating at a normal level during the entire period of two years of stay.

After tenth month, the reverse process started to manifest itself resulting in hyperglycemia. The hormonal or any other mechanism regulating these aspects of glucose metabolism need further investigation.

CONCLUSIONS

The exposure to high altitude of 4000 metres, constitutes a systemic stress on man. This stress is beyond the normal physiological limits of cardiorespiratory system to tackle with. This leads to hypoxic challenge at cellular and subcellular level causing adaptive changes to take place in tissues. The skeletal muscle hepatic, adrenal and renal tissues are pliable enough to modify themselves to face this challenge although this takes nearly 10-15 months in man at 4000 metres.

ACKNOWLEDGEMENTS

I am particularly grateful to Sarvshri Y. B. M. Rao, S. K. Bhardwaj, S. K. Grover, M. M. L. Kumria and A. Chander for helping me in carrying out the investigations. Surg. Commodore, M. S. Malhotra, Director, Defence Institute of Physiology and Allied Sciences, for his keen interest in the work, and Lt. Gen. Ved Prakash, DGAFMS and A.V.M. J. H. F. Manekshaw, DMR for permission to publish the work.

REFERENCES

1. GUYTON, A. C., "Basic Human Physiology" (W. B. Saunders Co., Philadelphia), 1971, p. 366.
2. SRIVASTAVA, K. K. & PRAKASH, O., *Def. Sci. J.*, **16** (1966), 125.
3. HAZUCHA, M., SILVARMAN, F., PARENT, C., FIELD, S. & BATES, D. V., *Arch. Environ. Health.*, **27** (1973), 183.
4. LUFT, U. C., "Phyiological Adaptations, Desert and Mountain", (Acad. Press. New York), 1972. p. 143.
5. RAHN, H. & OTIS, A. B., *Amer. K. Physiol.*, **157** (1949), 455.
6. VELASQUES, T., "School Aviation Med." (USAF. San. Antonio Texas Report No. 56-108), 1956.
7. WEST, J. B., LAHIRI, S., GILL, M. B., MILLEDGE, J. S., PUGH, L. G. C. E., & WARD, M. D., *J. Appl. Physiol.*, **17** (1962), 617.
8. LENFANT, C., TORRANCE, J. D., WOODSON, R.D., JACOBS, P. & FINCH, C. A., *Fed. Proc*, **29** (1970), 1115.
9. HARKNESS, D. R., *Adv. Intern. Med.*, **17** (1971), 189.
10. RAND, P. W., NORTON, J. M., BARKER, N. D., LOVELL, M. D., AUSTIN, W. H., *J. Appl. Physiol.*, **34** (1973), 827.
11. CHIODI, H., *Resp. Physiol.*, **11**, (1970-71), 84.
12. VALDIVIA, E., *Am. J. Phyiol.*, **194**, (1958), 585.
13. HURTADO, A., ROTTA, A., MERINO, C. & PONS, *J. Am. J. Med. Sci.*, **194** (1937), 708.
14. BULLARD, R. W. & KOLLIAS, J., *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, **25** (1966), 1288.
15. BUSIRK, E. R., "Biomedicine Problems at High Terrestrial Elevation" (Army Ins. Environ, Med.. Natick, Mass, USA), 1969. p. 323.
16. HURTADO, A., "Handbook of Physiology Sec. 4" (Williams & Wilkins, Baltimore, Maryland), 1964, p. 843.
17. DILL, D. B., *J. Amer. Med. Ass.*, **205** (1968), 747.
18. JOHNSON, H. L., CONSOLAZIO, C. E., DAWS, T. A. & KRZYWISKI, H. J., *Nutr. Rep. Intern.*, **4** (1971), 77.
19. BURRI, P. H. & WEIBEL, E. R., "High Altitude Physiology Cardiac and Respiratory Aspects., Ciba Symposium" (Churchill Livingston Edinburgh and London), 1971, p. 15.
20. ASMUSSEN, E., CONSOLAZIO, F. C., *Amer. J. Physiol.*, **132** (1941), 555.
21. EDWARDS, H. T., *Am. J. Physiol.*, **116** 1936, 367.
22. DILL, D. B., & ADAMS, W. C., *J. Appl. Physiol.*, **30** 1971, 854.
23. KLAUSEN, K., DILL, D. B., & HORVATH, S. M., *J. Appl. Physiol.*, **29** 1970, 456.
24. REYNAFARJE, B. & VELASQUEZ, T., *Fed. Proc. Fed. Amer. Soc. Exptl. Biol.*, **25** (1966), 137.
25. CUNNIGHAM, D. A., MAGEL, J. R., *Int. Z. Amgew. Physiol, Enschl. Arbeit, Physiol.*, **29** 1970, 94.
26. MENSENDE SILVA, E. & CAZORLA, A., *Am. J. Physiol.*, **224** (1973), 669.
27. CERRETELLI, P., "Exercise at Altitude," (R. Margaria, Excerpta. Med. Found., Amesterdam), 1967, p. 58.
28. PUGH, L. G. C. F., *Brit. Med. J.*, **2** (1962), 621-627.

29. CANSOLAZIO, C.F., JOHNSON, H. L. & KRYWICKI, H. J., "Physiological Adaptations, Desert & Mountain", (Acad. Press, New York and London), 1972, p. 227.

30. SRIVASTAVA, K. K., RAO, Y. B. M., BHARDWAJ, S. K., CHANDER, A. & MALHOTRA, M. S., *Aerospace Med.*, **44** (1973), 272.

31. SRIVASTAVA, K. K., RAO, Y. B. M., CHANDER, A. & MALHOTRA, M. S., *Ind. J. Exptl. Biol.*, **11** (1973), 130.

32. BHARDWAJ, H., *Human Biol.*, **44** (1972), 303.

33. REYNAFARJE, B., *J. Appl. Physiol.*, **17** (1962), 301.

34. OU, L. C. & TENNEY S. M., *Resp. Physiol.*, **8** (1970), 151.

35. BRAHMACHARI, H. D., MALHOTRA, M. S., JOSEP, S., RAMACHANDRAN, K., *Indi. J. Physiol. Pharmacol.*, **17** (1973), 365.

36. SRIVASTAVA, K. K. & MALHOTRA, M. S., *Inst. J. Biometereology*, **18** (1974).

37. RAMSOE, K., JARNUM, S., PREISIG, R., TAUBER, J., TYGSTRUP, N. & WESTERGAARD, H., *J. Appl. Physiol.*, **28** (1970), 725.

38. SURKS, M. I., *J. Clin. Invest.*, **45** (1966), 1442.

39. WESTERGAARD, H. S., JARNUM, S., PREISIG, R., RAMSOE, K., TAUBER, J., TYGSTRUP, N., *J. Appl. Physiol.*, **28** (1970), 728.

40. CHIODI, H. & SAMMARTINO, R., *Acta Physiol., Latinoam.*, **2** (1952), 228.

41. HANNON, J. P., CHINN, K. S. K., & SHIELDS, J. L., *Fed. Proc.*, **28** (1969), 1178.

42. KLAIN, G. J. & HANNON, J. P., *Proc. Soc. Exptl. Biol. Med.*, **129** (1968), 648.

43. HETHERINGTON, A. W., LUFT. U. & IVING, J. H., *Digst. Ser.*, **4** (1947), G.E. 61/1, Dec.

44. RAI, R. M., MALHOTRA, M. S., DIMRI, G. P. & SAMPATHKUMAR, T., *Am. J. Clin. Nutr.*, 1974 (in pres)

45. RENNIE, D., FRAYSER, R., GARY, G. & HOUSTON, C., *J. Appl. Physiol.*, **32** (1972), 369.

46. RENNIE, D., MORTICORENA, E., MONGE, C. & SIROTZKY, L., *J. Appl. Physiol.*, **31** (1971), 257.

47. RENNIE, I. D. B. & JOSEPH, B. J., *Lencet.*, **1** (1970), 1247.

48. MONCLOA, F., CARCELEN, A. & BETETA, L., *J. Appl. Physiol.*, **28** (1970), 151.

49. CUNNIGHAM, W. L., BECKER, E. J. & KREUZER, F., *J. Appl. Physiol.*, **20** (1965), 607.

50. HORNBEIN, T. F., *J. Appl. Physiol.*, **17** (1962), 246.

51. HALHUBER, M. J., & GABLE, F. "The Physiological Effect of High Altitude". (Pergamon Press, Oxford) 1964. p. 131.

52. KLEIN, KARL-EGON., *Ibid.*, (1964), 136.

53. CRISCULO, D., HALE, H. B. & MEFFERED Jr., R. B., *J. Appl. Physiol.*, **13** (1958), 353.

54. SIRI, W. E., CLEVELAND, A. S. & BLANCHE, P., *Fed. Proc.*, **28** (1969), 1251.

55. MAC INNES, C., ROTHWELL, R. I., JACOBS, H. S. & NABARRO, J. D. N., *Lancet* **1** (1971), 49.

56. PUGH, L. G. C. E., "The Scientific Basis of Medicine, (Reviews) 1962, 32.

57. MONCLOA, F. & PRETELL, E., *J. Clin. Endocrinol. & Metab.*, **24** (1964), 915.

58. VAUGHAN, D. A., STEELE, J. L. & KORTY, P. R., *Fed. Proc.*, **28** (1969), 1110.

59. PICON-REATEGUI, E., *J. Appl. Physiol.*, **21** (1966), 1117.

60. BLUME, F. D. & PACE, N., *J. Appl. Physiol.*, **23** (1967), 75.

61. CALDERON, R., LLERANA, L. A., MINIVE, L. & KRUGER, F., *Dibites.*, **15** (1966), 130.

62. DAVIDSON, M. B., *J. Appl. Physiol.*, **25** (1968), 105.

63. FORBES, W. H., *Am. J. Physiol.*, **116** (1936), 309.

64. BRAHMACHARI, H. D., MALHOTRA, M. S., JOSEH, S. & RADAKRISHNAN, U., *Ind. J. Med. Res.*, **61** (1973), 411.