[The discovery of carrier proteins for water-soluble vitamins in mammalian and avian species by Professor P. R. Adiga and his colleagues, has evoked considerable interest among biochemists. These proteins transport across the placenta, specific vitamins from the mother to the developing embryo. It is for this work that Prof. Adiga received the Shanti Swarup Bhatnagar prize. This work is summarized in this invited article.—Ed.]

TRANSPORT OF WATER-SOLUBLE VITAMINS IN PREGNANCY

N. APPAJI RAO

Department of Biochemistry, Indian Institute of Science, Bangalore 560 012

ONE of the intriguing problems in vertebrate reproduction is the mechnaism by which the developing embryo concentrates for its growth the essential nutrients such as vitamins from the maternal system in which these nutrients are present in low concentrations. A significant development in the understanding of this problem has been the isolation of the unique carrier proteins which bind themselves to the vitamins in the maternal system in a highly specific manner and transport them across the placenta to the growing embryo.

Work in this area was initiated a few years ago in the laboratory of Professor P. R. Adiga at the Indian Institute of Science, Bangalore. Professor Adiga's approach to the problem was to use chicken as a model system and search for highly specific proteins within the egg which bind themselves to riboflavin and thiamin. The choice of this model system was most appropriate because in the avian system, all the nutrients required for the growing embryo are stored within the egg. In the mammalian system, on the other hand, the foetus continuously obtains its requirements for growth from the mother. These binding proteins were isolated by conventional methods as well as by using novel affinity chromatographic procedures. In the latter methods, the vitamin is hooked to an immobile matrix such as sepharose or Agarose and a solution containing the carrier protein, stripped of its vitamin is passed through the affinity matrix. When the protein encounters the immobile vitamin, it interacts specifically with the vitamin and is thus retained on the column. After washing the other proteins from the column, the carrier proteins are isolated by eluting them with a solution of high concentration of the vitamin which by competition releases the carrier protein from the matrix. An interesting feature of these carrier proteins is that they bind strongly to the free vitamin but have very weak affinities for its coenzymic forms. It is worthwhile to recall here that the physiological role of the water-soluble vitamins in most instances is as coeznymes which, as the components of specific enzymes, catalyse a variety of chemical reactions. These proteins represent, therefore, a new category which interacts with free vitamins but have no known catalytic function.

Professor Adiga hypothesized that these proteins are present in the egg to meet the needs of the growing embryo because the

vitamins are essential for normal embryonic development. Employing these carrier proteins, antibodies were raised in the rabbit, and highly specific and sensitive radioimmuno assays were standardized to detect these vitamin-carrier proteins in the maternal circulation. This assay enabled the detection of these proteins in the maternal system and also to establish the relationship between the hormonal status of the mother during pregnancy and estrus as well as to estimate the concentration of these vitamin-carrier proteins in circulation. A cause-effect relationship between pregnancy and the presence of these vitamin-carrier proteins in the maternal circulation was thereby established.

Antibodies raised against the chicken vitamin binding proteins cross-reacted and completely neutralized a similar protein present in the serum of pregnant rats. This result suggested that vitamin-carrier proteins immunologically similar to those present in the chicken were also present in the pregnant rat. Later researches resulted in the isolation, for the first time, of the rat riboflavin and thiamin-binding proteins. When pregnant rats were administered antisera, raised against vitamin-binding proteins isolated either from rat or chicken, the growing foetus was resorbed or aborted. This observation is of great significance to our understanding of reproductive physiology and foetal nutrition. These results become important when examined in the context of the well-documented phenomenon of the preferential accumulation of the B group of vitamins by the developing foetus, even though the placenta is impermeable to free vitamins as well as to their coenzymic forms. This accumulation of vitamins by the growing embryo even when the mother is marginally deficient in vitamin status suggests that the foetus is an autonomous system selectively demanding, in obtaining its requirements from the maternal circulation.

When pregnant rats were administered antisera raised against carrier proteins, either alone or along with the vitamins, resorption or abortion of the foetus occurred even though the vitamin levels in the maternal circulation were adequate. These observations indicated that the foetus depended on a different system for the transport and utilization of these vitamins and that transplacental transport of these vitamins was mediated by specific carrier proteins. Recent unpublished work suggests that transport of radioactively labelled vitamin to the foetus from the maternal circulation is completely inhibited when antibodies to the carrier proteins are administered along with labelled vitamins. Injection of chicken riboflavin- or thiamin-carrier proteins into normal adult female rats resulted in the production of antibodies to these proteins but had no effect on their normal estrus cycle nor did it cause vitamin deficiency in them. These rats, when mated, conceived normally but when the placenta became established around the 10th day of pregnancy, foetal wastage and resorption occurred. As long as the carrier antibody levels were high, these animals conceived normally but could not proceed through the complete term of pregnancy. When the antibody levels decreased with time to near undetectable levels, the pregnancy proceeded to completion. The pups were normal with no signs of teratogenic abnormalities or vitamin deficiency. These observations demonstrate the important role the carrier proteins play in the maintenance of pregnancy and in foetal well-being. Professor Adiga has also shown that similar carrier proteins are present in the serum of other mammals like the monkey and the human, suggesting that similar mechanisms of vitamin transport during pregnancy may be operating in these higher species as well.

These proteins provide a facile tool for examining the maternal-foetal relationship and the role of the placental barrier in this relationship. These results also show that in addition to a satisfactory vitamin status of the mother, specific delivery systems ultimately

determine the vtamin nutrition of the foetus and hence its development. Professor Adiga's observations may provide a possible explanation for some of the ill-defined cases of habitual abortions and teratogenesis. Some of these cases may be due to genetic and endocrine defects in the production and/or functionality of these vitamin carriers. The induction of these reproduction-specific carrier proteins, by estrogen suggests for the first time a possible function for this hormone, which is present in high concentrations throughout pregnancy, in foetal development. The removal of these proteins from the maternal circulation by interaction with specific antibodies may be used as a promising and useful new approach to medical termination of pregnancy and contraception.

In conclusion the most significant findings are : the discovery of specific carrier proteins for the water-soluble vitamins and their function in the transport of these vitamins across the placental barrier to the foetus; the evidence that these proteins are specifically induced by the sex hormone, estrogen, by interacting with the maternal genome during pregnancy; evidence that specific neutralization of these proteins leads to foetal wastage without affecting or interfering with the maternal well-being; and finally, the possibility of using antibodies to these proteins as a new approach to control fertility.

DETECTION OF HUMAN CARDIAC ACTIVITY AT APEX REGION BY LASER SPECKLE

A. PERIASAMY, MEGHA SINGH AND B. M. SIVARAM*
Biomedical Engineering Division, Indian Institute of Technology, Madras 600 036, India

ABSTRACT

Time average speckle interferometric technique has been used to obtain the displacement pattern over the chest rgion of healthy subjects. It shows that the displacements, over the apex region, caused by the heart movement, differ significantly from that of the corresponding region on the right side of the chest.

INTRODUCTION

WHEN an optically rough surface is illuminated by a coherent light as that from a laser, the surface has a grainy or speckled appearance. The speckles, which are due to an interference phenomenon, are localized in space and move with the movement of the eye.

Leser speckle interferometry provides a non-contact technique which has been used for various engineering applications such as for measuring displacement, strain and the roughness of plane surface[1–5]. The application of laser speckle in medicine is still in infancy. Zivi *et al.*[6] using double pulsed ruby laser holography observed the patterns of motion of the surface of a human chest during inhalation. Hok *et al.*[7] applied a similar technique for studying the patterns of chest wall motion due to heart action. But these techniques provide only a qualitative information of the chest wall movement. The present technique is much simpler than the ruby laser holographic technique and provides a quantitative assessment of the displacement pattern over the chest wall caused by the cardiac movement.

METHOD

The recording of time average specklegram was carried out in the Fourier transform plane [Fig. 1 (*a*)]. A healthy subject was asked to sit in an upright position on a chair. For recording the speckle pattern, the subject was asked to inhale and to exhale and then to hold the breath, so that during the recording of the chest movement the abdomen wall did not suffer any displacement. To overcome the body movements during the experiment the subject was firmly fastened to the chair with a belt.

A 5·0 mw He–Ne laser (Polytec—Germany) of wavelength 632·8 nm was used as a light source. The beam was expanded by a beam expander to illuminate an area over the apex region on the left side of the chest. A camera loaded with Agfa-Gevaert Scientia 10E75 photographic plate was placed in the Fourier plane of a large aperture convex lens of focal length 15·0 cm [Fig. 1 (*a*)]. The heart beat of the subject was approximately 70 beats/min. Accordingly the exposure time was of the order of 5 seconds, as for time average speckle interferometry the exposure time should be longer than one second. This plate was processed using D19 developer and DA163 fixer.

Using the same technique, another speckle pattern to compare with that of the apex region was recorded on the right side of the chest wall. The distance of the region from the midline was the same as that of the apex region on the left side of the chest.

The recorded speckle pattern was analyzed by the point-wise method [Fig. 1 (*b*)]. The speckle interferogram was mounted on an upright, provided with hori-

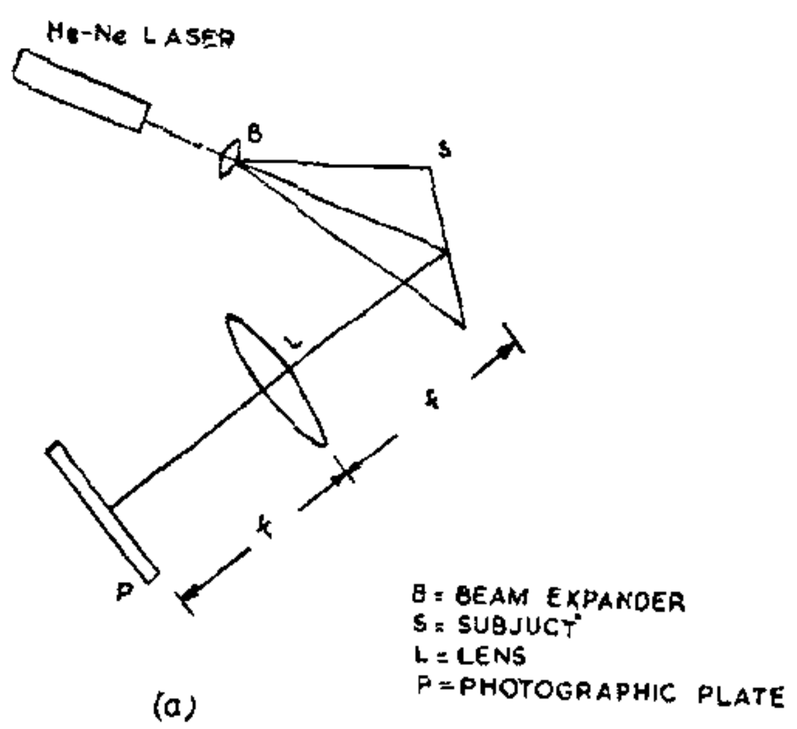

FIG. 1 (*a*). Schematic of the experimental arrangement to obtain speckle interferogram (specklegram). (*b*) Schematic for the analysis of speckle interferogram

* Department of Physics,