

following hormone treatment. The probable mechanism of influence of testosterone on the adrenal cortex is discussed.

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ELECTROPHORETIC FRACTIONATION OF SERUM PROTEINS IN EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS.

BY

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CHAKRAVARTI, DE and MUKERJI (1956) reported relative serum hyperglobulinæmia with reversal of A/G ratio in experimental cholesterol atherosclerosis of rabbits. Dubach and Hill (1946) showed the same phenomenon in sustained hypercholesterolaemia of rabbits and Fishberg *et al.* (1950) described beta-hyperglobulinæmia as a result of prolonged cholesterol feeding. The significance of serum proteins, chiefly the globulin fractions, lies in the fact that serum lipids exist in combination with proteins as lipoprotein complexes, the state and relative concentrations of which determine the development of atheroma, both in humans and in experimental animals (Sacks, 1951). Therefore, in this work changes in serum-protein fractions occurring in experimental cholesterol atherosclerosis in rabbits have been studied.

MATERIALS AND METHODS.

Male rabbits, six in number, of the C.D.R.I. colony, average weight 1.5 kg., were fed 0.5 g. of cholesterol (Merck) in 5 c.c. of olive oil daily. Twelve weeks later the animals were sacrificed and the diagnosis of atheroma established by gross and microscopic examination.

Blood was collected before and at 4, 8 and 12 weeks of feeding cholesterol, respectively. The electrophoresis of different sera was carried out on Whatman No. 3 mm. filter-paper strips, 30 x 250 mm. in size. Veronal buffer pH 8.6 μ 0.1 was used for filling electrode vessels and wetting filter paper strips. In each estimation 0.01 c.c. of serum was used. The tests were performed in duplicate. The apparatus was run for 16 hours at 110 volts and 2 ma current for each filter paper. The filter papers were then dried under an infra red lamp and dipped for

16 hours in aqueous bromophenol blue bath containing mercuric chloride. After staining the strips were rinsed with 2 per cent acetic acid solution for 5, 5 and 10 minutes, respectively, and finally for 3 minutes with 2 per cent sodium acetate and 10 per cent acetic-acid solution. The strips were blotted and dried for 30 minutes. The readings were taken on a photovolt direct reading densiometer, and plotted. The values of the protein fractions were calculated by determining the area under the curve.

TABLE.

Mean values of serum proteins in six rabbits before and after cholesterol feeding.

Duration of cholesterol feeding.	Total proteins.	Albumin.	GLOBULINS:			
			Alpha 1.	Alpha 2.	Beta.	Gamma.
Base	7.0±0.6	55.4±4.0	3.7±0.9	3.8±0.6	12.9±1.4	24.2±3.1
After 4 weeks	7.5±0.9	52.0±7.0	4.7±2.2	6.3±3.4	13.7±2.4	22.3±4.2
After 8 weeks	8.2±0.3	46.9±4.6	6.1±3.1	9.1±3.3	15.4±2.3	22.5±3.1
After 12 weeks	7.5±0.6	48.5±4.2	3.4±1.3	6.7±1.9	14.7±2.7	26.7±5.0

RESULT.

From the Table and Graphs 1 and 2 the following can be deduced:—

Total proteins.—A sustained rise from the base value is seen in case of total serum proteins which is maintained throughout the period of cholesterol feeding and attains a maximum at eight weeks.

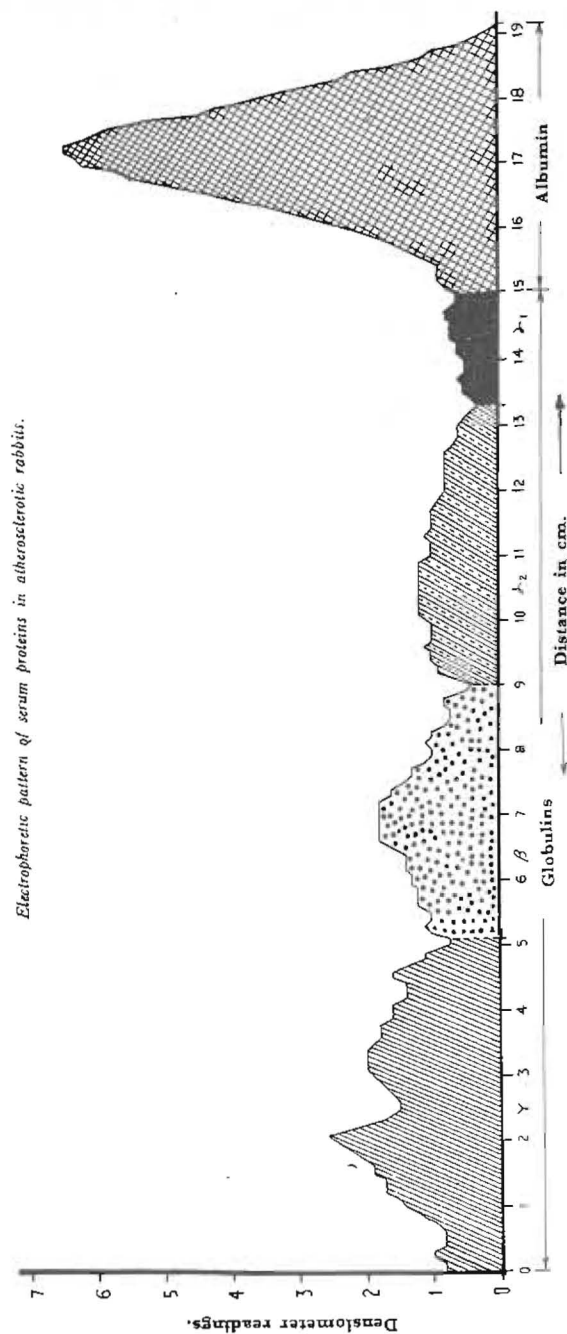
Albumin.—Albumin records a gradual decrease from the base value and at the end of twelve weeks a 13 per cent drop from the normal is seen.

Globulins.—The alpha 1 globulin shows 30 per cent increase from the base value at fourth week followed by an increase of another 39 per cent at eighth week. At the twelfth week period there is, however, a steep fall (45 per cent) which brings down the value lower than the base. The alpha 2 globulin shows a gradual rise, 21 per cent increase at the fourth week and attaining its maximum at eighth week registering a 75 per cent increase from the base value. At the twelfth week period, however, the mean value remains only 29 per cent higher than the base. The beta globulin also increases gradually from the base value, 6 per cent increase after four weeks of feeding, and attains a maximum (20 per cent) at eighth week. At twelfth week there is, however, decrease from that of the eighth week but still remains 14 per cent higher than the base.

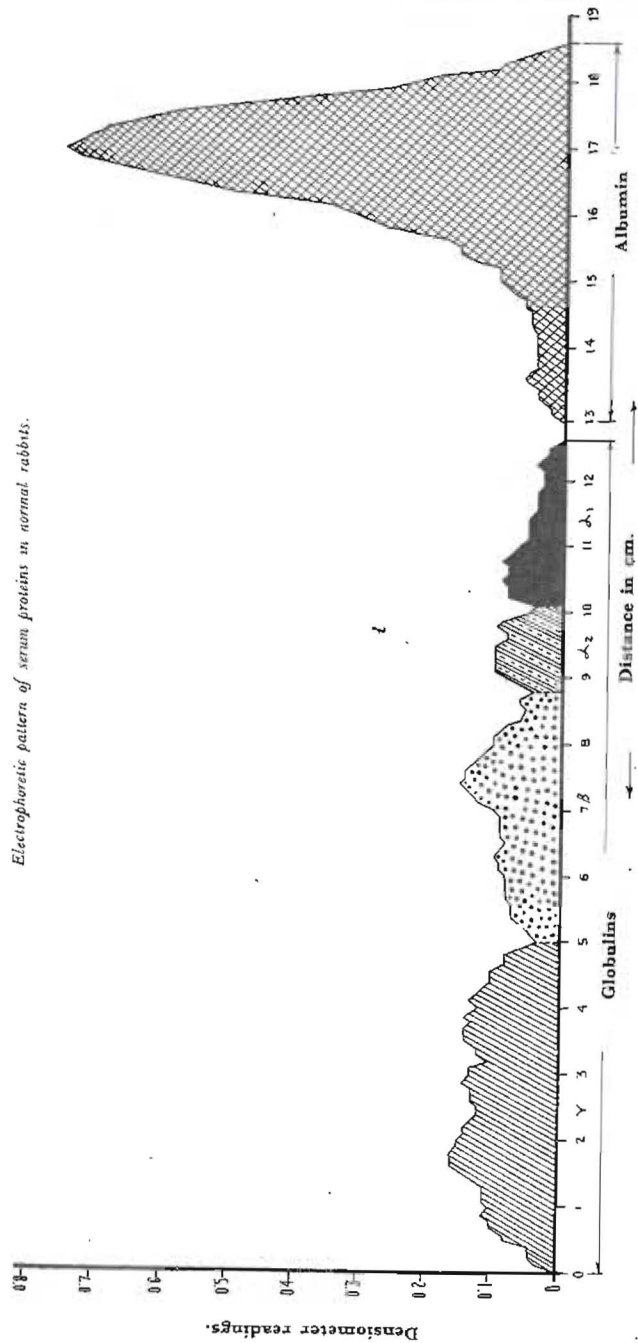
The gamma globulin shows 3.6 per cent fall from the base value at the fourth week, another 2.4 per cent at eighth week but goes up again attaining a value 10 per cent higher than the base at the twelfth week period.

A/G ratio.—The albumin : globulin ratio is reversed at eighth and twelfth week periods, registering a value of less than unity.

GRAPH 1.



GRAPH 2.
Electrophoretic pattern of serum proteins in normal rabbits.



DISCUSSION.

From the data obtained in this experiment, it will be evident that prolonged administration of cholesterol in oil to rabbits results in atheromatous lesions leading to a relative serum hyperglobulinæmia with reversal of A/G ratio. This confirms our previous findings (Chakravarti, De and Mukerji, *loc. cit.*).

Among the globulin fractions, there is an overall increase except alpha 1 globulin at the end of twelve weeks, although sharp fluctuations are observed in the intermediate period. The alpha 1 globulin shows an initial rise followed by a marked fall at the end of twelfth week, while the alpha 2 and beta globulins continue to increase throughout. Again, alpha 1, alpha 2 and beta globulins attain maximum values at eighth week which is consistent with a similar increase in the total proteins. On the contrary, there is relative decrease of albumin and gamma globulin fractions at the early phases although the latter increases at the twelfth week period.

Other workers (Fishberg *et al.*, *loc. cit.*; Antonini *et al.*, 1953; and Soulier *et al.*, 1953) also found a similar protein picture, a relative hyperglobulinæmia with marked increase of alpha 2 and beta globulins in atherosclerosis, but these studies did not demonstrate the changes that occur at different periods of development of atheroma. The interesting finding in this experiment is that all the protein fractions of serum (except fibrinogen which has not been studied) show maximum change, either increase or decrease, at the eighth week of feeding cholesterol. In a previous experiment Chakravarti, De and Mukerji (1955) showed that definite histological changes in the aorta occur as early as five weeks of feeding cholesterol to rabbits and that precedes the deposition of lipid in the aortic intima. This is amplified by our present experiment which points out to a critical physico-chemical imbalance in the blood, and may also be in tissue, at this stage when typical atheroma is not developed.

Again, it has been emphasized by different workers (Gofman *et al.*, 1951; Antonini *et al.*, *loc. cit.*; Gottfried *et al.*, 1955; French, 1955) that the state and relative concentration of lipoprotein complexes in the system determine the development of atheroma in an individual and alpha 2 and beta lipoproteins are important in this respect. We have also found that these two protein fractions are relatively increased. Further studies in the picture of lipoproteins of serum in atherosclerotic subjects will possibly be able to clarify the relationship between serum lipids and proteins and thereby throw some light in the understanding of the pathogenesis of atherosclerosis.

SUMMARY.

1. Fractionation of serum proteins has been performed by paper-electrophoresis in atherosclerotic rabbits at different periods of cholesterol feeding.

2. A relative serum hyperglobulinæmia of alpha 2, beta and gamma fractions with reversal of A/G ratio has been obtained at the termination of the experiment. The alpha 1 globulin shows an initial rise but falls below the normal level at twelve weeks.

3. Maximum changes in the serum proteins occur at the eighth week stage developing a critical physico-chemical alteration in the system which may precipitate the atheromatous change.

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SIGNIFICANCE OF MULTIPLE PATHWAYS IN ADAPTATION.

PART II.

INHIBITION OF GERMINATION OF *A. NIGER* AS INDUCED BY EXPOSURE TO PHENYLMETHANE DYES.

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It has been shown in a previous communication (Bose, 1955) that a vital process like sporulation of a strain of *A. niger* follows more than one biochemical path. This is taken as an evidence to indicate that cells to adapt in response to a given stimulus must have multiple pathways for all its vital reactions (Bose, 1956). Drug adaptation has been accounted for by the development of an alternate mode of growth (Hinshelwood, 1951). It is quite conceivable that germination, another vital reaction in the life process of an organism, may have multiple routes.

The present work was, therefore, undertaken to investigate whether or not multiple pathways are really involved in germination. For this purpose a study has been made of the effect of phenylmethane dyes on the period of germination of a given organism and also on the pattern of inhibition induced therein.

METHODS AND MATERIALS.

(a) *Organism and media.*—The organism used was a strain of *A. niger* G₃Br. It was maintained on an agar medium consisting of glucose 50 g., sodium nitrate 2.0 g., potassium dihydrogen phosphate 1.0 g., magnesium sulphate 0.5 g., yeast extract 0.5 g., malt extract 0.5 g. and potato extract (hot water extract of 100 g. of potatoes) per litre of the medium. This medium was also used for determining the period of germination, and for harvesting of spores, the same medium was used except without the addition of agar.

(b) *Method of obtaining conidial suspension free from vegetative growth, technique of exposure to dyes, etc.*—These were detailed in an earlier publication (Mazumdar and Bose, 1955).