Caucasians has been reported previously (for references, see Kirk *et al.* 1960).

Note added in proof. Since submitting this paper, we have observed that both caeruloplasmin and a globulin–haem complex (see Nyman, 1960) react with the dianisidine reagent used to detect the haptoglobin–haemoglobin complex after paper electrophoresis. Although caeruloplasmin migrates clearly ahead of the haptoglobin–haemoglobin complex on paper electrophoresis at pH 6·5, the globulin–haem complex and the haptoglobin–haemoglobin complex do not separate under these conditions. Accordingly the finding of a dianisidine reactive area in the α_2-globulin zone (pH 6·5) does not necessarily indicate the presence of the haptoglobin–haemoglobin complex. Since some of the 800 normal Caucasian sera examined by paper electrophoresis were found to contain methaemalbumin, it is possible that they also contained the globulin–haem complex. Consequently our findings do not necessarily exclude a haptoglobinaemia in these sera.

SUMMARY

1. The peroxidase activities of methaemoglobin and of methaemoglobin–haptoglobin complex on the reaction between guaiacol and hydrogen peroxide have been examined and a method for the determination of haptoglobins in serum was devised.

2. The method has been used to determine the serum haptoglobin concentration in normal human serum. A mean value (as bound methaemglobin) of 93 mg./100 ml. (S.D. 40) was obtained. The mean serum-haptoglobin concentration varied according to the genetic haptoglobin type.

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The Glycolytic Enzymes of Guinea-Pig Lung in Experimental Bagassosis

BY V. N. SINGH, T. A. VENKITASUBRAMANIAN AND R. VISWANATHAN
Vallabhbhai Patel Chest Institute, Delhi University, Delhi, India

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In a previous study of experimental bagassosis Singh, Mitra, Venkitasubramanian & Viswanathan (1960) showed that in this condition there was an appreciable change in the activity of various respiratory enzymes and in the Q_{O_2} of guinea-pig lung. Also, V. N. Singh, T. A. Venkitasubramanian & R. Viswanathan (unpublished work) found a remarkable alteration in the chemical composition of

lung. These results suggest that there may be a metabolic derangement in guinea pigs exposed to bagasse dust. The latter study demonstrated, together with other alterations, an increase in the glycogen content of guinea-pig lung as a result of experimental bagassosis.

It might be expected that a study of the enzyme systems involved in the synthesis and breakdown of glycogen would contribute to an understanding of the mechanism of metabolic response of guinea pigs to experimental bagassosis. To obtain an insight into the mechanism of glycogen accumulation a study of certain important enzymes of the glycolytic pathway, namely phosphorylase, hexokinase, phosphoglucoisomerase and aldolase, as well as the estimation of lactic acid in lung tissue of guinea pigs exposed to bagasse dust, has been made.

MATERIALS AND METHODS

Chemicals. Glucose 1-phosphate, adenosine triphosphate, glucose 6-phosphate and fructose-1:6-diphosphate were all Sigma Chemical Co. products.

Fructose 1:6-diphosphate as the barium salt was dissolved in N-HCl and freed from barium by addition of the required amount of Na_2SO_4. The slight yellow colour of the solution was removed by shaking with Norit charcoal. The solution was adjusted to pH 8·6 by the addition of NaOH, filtered, and the concentration of fructose diphosphate in the solution was determined by estimating fructose by the method of Roe (1934). The solution was then diluted to give a concentration of 0·05 M.

Lactic acid (85 %) was diluted to approx. 1·0 M, slightly over-neutralized with NaOH, boiled, and then back-titrated and diluted appropriately.

Animals. Young guinea pigs (300–500 g.) were selected and paired for weight, age and sex. They were fed without restraint. One guinea pig of each pair was kept as a control, and the other one was exposed to bagasse dust by a mechanical device (Singh *et al.* 1960) for various periods of time as described in the Results section.

Tissue collection. Animals were stunned by a blow on the head and decapitated. Lung tissue was removed as rapidly as possible, freed from adhering blood with filter paper and kept in a container cooled in an ice–salt mixture. Lung tissue was used for the various estimations as quickly as possible.

Enzyme assays

Phosphorylase. The methods of Shull, Ashmore & Mayer (1956) and of Cori, Cori & Green (1943) were modified to make the procedure applicable to lung. The lung tissue was homogenized in 0·1 M-NaF to give a 15 % (w/v) homogenate. Fluoride served the purpose of inactivating phosphoglucomutase (Najjar, 1948), which would otherwise interfere with the estimation by converting glucose 1-phosphate into glucose 6-phosphate. Suitable amounts of homogenate were pipetted into graduated centrifuge tubes containing 0·7 ml. of 0·154 M-NaF, 0·2 ml. of 0·25 M-glucose 1-phosphate, 0·6 ml. of 0·1 M-citrate buffer (pH 5·8), 0·1 ml. of 4 % (w/v) glycogen, and the volume was made up to 2·0 ml. with water. The reaction mixture was incubated for 30 min. at 30° and the reaction was then stopped by the addition of 1·0 ml. of ice-cold 10 % (w/v) trichloroacetic acid. The volume was made up to 10·0 ml. and after the tubes were kept in an ice bath for 20 min. they were centrifuged, and the solution was filtered through Whatman no. 42 filter paper. The filtrate was taken for the estimation of inorganic phosphorus by the method of Sumner (1944) with slight modifications. To 2 ml. of the filtrate, taken in a test tube and made up to 8·2 ml. with water, 0·5 ml. of 6·6 % (w/v) ammonium molybdate, 0·5 ml. of 7·5 N-H_2SO_4 and 0·8 ml. of freshly prepared 10 % (w/v) $FeSO_4$ (reducing agent) were added. Standard phosphorus (15, 30 and 45 μg.) controls were also run in each experiment. The extinction was read after 10 min. at 660 mμ in a Bausch and Lomb colorimeter.

For each concentration of homogenate a blank was prepared with the enzyme inactivated at zero time.

Hexokinase. Activity was estimated by the method of Long (1952) with 25 % (w/v) lung homogenate. Because of the inhibitory action of Na^+ ions on hexokinase activity (Wiebelhaus & Lardy, 1949) an all-potassium medium as recommended by Long (1952) was used. The incubation period was 10 min. Glucose was estimated by the Somogyi (1952) method as recommended by Kerly & Leaback (1957). For each concentration of the homogenate a separate blank was run with the enzyme inactivated at zero time. For every experiment a reference standard curve of glucose was prepared.

Phosphoglucoisomerase. The method of Glock, McLean & Whitehead (1956) based on that of Slein (1955) was followed for the assay of the enzyme with 0·2 % (w/v) tissue homogenate. The fructose 6-phosphate formed was determined colorimetrically by the method of Roe (1934), the extinction being read at 490 mμ in a Bausch and Lomb colorimeter. Experimental details were those described by Glock *et al.* (1956) except that 0·05 M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer, pH 7·6, replaced glycylglycine buffer. For each concentration of homogenate a blank was run where enzyme activity was stopped at zero time. The concentration of fructose 6-phosphate was determined by referring to a standard fructose solution which was analysed with every experiment.

Aldolase. Activity of this enzyme was estimated by the method of Sibley & Lehninger (1949) by using 2 % (w/v) tissue homogenate. The homogenate was brought to 38° before addition to the reaction mixture, as suggested by Swenson & Boyer (1957). The enzyme was inactivated at the end of the incubation period of 15 min. by addition of 10 % (w/v) trichloroacetic acid as recommended by Beck (1955), and the tubes were kept in ice for 10 min. (Roodyn, 1956). After addition of 2:4-dinitrophenylhydrazine the tubes were incubated at 38° for 30 min. (Beck, 1955) for colour development.

For each homogenate concentration a blank was prepared in which the homogenate was added only after the addition of trichloroacetic acid. The extinction was read at 540 mμ, and results were calculated by referring to a standard curve (Sibley & Lehninger, 1949).

Determination of lactic acid. The method of Barker & Summerson (1941) was followed. As soon as the animal was dissected a small portion of the lung tissue was freed from adhering blood with filter paper and transferred to weighed 15 ml. centrifuge tubes containing 2 ml. of 10 % (w/v) trichloroacetic acid. The tubes were reweighed to obtain the weight of the tissue. Trichloroacetic acid was added to

Table 1. *Effect of experimental bagassosis on phosphorylase, phosphoglucoisomerase, hexokinase and aldolase activity of guinea-pig lung*

Details of the assay procedures are given in the Methods section. Values are given as means ±S.E. (with number of experiments shown in parentheses).

Time of exposure to dust (hr.)	(*a*) Phosphorylase: Inorganic phosphorus liberated (μg. atoms/g. wet wt./min.)	(μg. atoms/g. dry wt./min.)
Nil	2·59±0·06 (9)	12·23±0·52 (9)
44–114	2·46±0·07 (9)	12·81±0·58 (9)
	$0{\cdot}2 > P > 0{\cdot}1$	$0{\cdot}5 > P > 0{\cdot}4$
	(*b*) Phosphoglucoisomerase: Fructose 6-phosphate liberated	
	(μmoles/g. wet wt./min.)	(μmoles/g. dry wt./min.)
Nil	90·8±1·6 (6)	429·8±12·1 (6)
51–68	133·8±2·1 (6)	682·5±14·6 (6)
	$P < 0{\cdot}001$	$P < 0{\cdot}001$
	(*c*) Hexokinase: Glucose used	
	(μmoles/g. wet wt./min.)	(μmoles/g. dry wt./min.)
Nil	0·33±0·03 (7)	1·38±0·07 (7)
51–63	0·67±0·06 (7)	3·81±0·64 (7)
	$P < 0{\cdot}001$	$P < 0{\cdot}005$
	(*d*) Aldolase: Hexose diphosphate used	
	(μmoles/g. wet wt./min.)	(μmoles/g. dry wt./min.)
Nil	3·26±0·07 (8)	12·83±0·26 (8)
52–63	3·94±0·22 (8)	18·37±1·01 (8)
	$0{\cdot}02 > P > 0{\cdot}01$	$P < 0{\cdot}001$

minimize the error due to glycolysis. The tissue was homogenized and diluted to give a concentration of 0·4% (w/v) of homogenate. A reagent blank was run through the complete procedure and a standard curve for lactic acid was prepared every time.

RESULTS

Results of the enzyme assays are given in Table 1. In studying phosphorylase activity, experimental animals were exposed to the bagasse dust for periods varying from 44 to 114 hr. Nine animals from each group, control and experimental, were used for the assay. As can be seen from the Table, experimental bagassosis does not induce any significant change in the phosphorylase activity of guinea-pig lung, although the trend, if any, seems to be towards a decrease.

Hexokinase activity is reported in the same Table. Experimental animals used for the assay of this enzyme were exposed to bagasse dust for periods varying from 51 to 63 hr. It is evident that hexokinase activity of guinea-pig lung is significantly increased in experimental bagassosis.

Results of the estimation of phosphoglucoisomerase show that the activity of this enzyme in guinea-pig lung was remarkably enhanced in bagassosis.

Aldolase activity is also given in Table 1. It is evident that aldolase activity of guinea-pig lung is significantly increased in bagassosis.

Results on the lactic acid content of lung are given in Table 2. The results are expressed in terms of (i) μmoles of lactic acid/g. of wet tissue, (ii) lactic acid (μmoles) of whole lung tissue/100 g. body wt. and (iii) total lactic acid (μmoles) in the whole lung, which will be referred to as total lactic acid. As can be seen from the Table, the lactic acid content of guinea-pig lung was significantly increased in bagassosis.

DISCUSSION

As the moisture content of guinea-pig lung is increased by exposure to bagasse dust, it was thought desirable to express the results both as per g. of wet tissue and per g. of dry tissue. Conversion of the results on wet tissue basis to those on dry

Table 2. *Lactic acid content of lung tissue of normal guinea pigs and those exposed to bagasse dust*

For experimental details see the Methods section. Figures of results are means ±S.E. with number of experiments shown in parentheses.

	Normal guinea pigs	Experimental guinea pigs (exposed to dust 41–115 hr.)	Significance of difference
Lactic acid (μmoles/g. of wet tissue)	10·9±0·72 (9)	14·7±1·3 (9)	$0·02 < P < 0·025$
Lactic acid (μmoles/100 g. body wt.)	8·8±0·46 (9)	16·7±2·0 (9)	$0·001 < P < 0·005$
Total lactic acid (μmoles/whole lung)	38·3±2·8 (9)	70·3±9·2 (9)	$0·001 < P < 0·005$

tissue basis appears to give a different ratio for normal tissue on one hand and experimental tissue on the other. This can be explained by the increased moisture content of guinea-pig-lung tissue in experimental bagassosis.

The discovery of Leloir & Cardini (1957) of an enzyme in liver which brings about uridine diphosphate glucose-linked synthesis of glycogen and the subsequent studies of this enzyme in several other tissues (Leloir, Olavarria, Goldemberg & Carminatti, 1959; Villar-Palasi & Larner, 1960) have stimulated further extensive studies in this field. Considerable evidence has recently accumulated suggesting that under physiological conditions the uridine diphosphate glucose-linked pathway serves an anabolic function whereas phosphorylase plays a catabolic role in glycogen metabolism (Sutherland, 1956; Robbins & Lipmann, 1959; Schmid & Mahler, 1959; Schmid, Robbins & Traut, 1959; Larner & Villar-Palasi, 1959). Thus an almost unaltered phosphorylase activity of guinea-pig lung in experimental bagassosis suggests that there is no appreciable change in the catabolic phase of glycogen metabolism.

When the role of hexokinase in diseases of carbohydrate metabolism such as diabetes (Price, Cori & Colowick, 1945; Cori, 1945–46; Hoffman, 1949; Chernick, Chaikoff & Abraham, 1951) is considered, the increase in the activity of this enzyme in guinea-pig lung in experimental bagassosis is of interest. Increased hexokinase activity will augment the phosphorylation of glucose and thereby increase the glucose 6-phosphate pool of the diseased guinea-pig lung. The increased glucose 6-phosphate could be channelled towards the synthesis of glycogen as well as towards the glycolytic pathway which leads finally to the production of lactic acid or pyruvic acid, depending on the supply of oxygen.

The activities of phosphoglucoisomerase and aldolase of guinea-pig lung are also increased in experimental bagassosis and thus the rates of the following reactions will be accelerated:

glucose 6-phosphate ⇌ fructose 6-phosphate

and

fructose 1:6-diphosphate ⇌ triose phosphates.

At present it is not possible to decide whether the increase in these two enzymes is a primary effect of the disease or a secondary effect of the increased glucose 6-phosphate pool.

The elevated lactic acid content of guinea-pig-lung tissue in experimental bagassosis is also of interest. Hypoxia, which is induced by experimental bagassosis in guinea-pig lungs, would lead to the predominance of anaerobic glycolysis in order to cope with the demand for oxidized pyridine nucleotides and with the requirement of energy, thereby augmenting the production of lactic acid.

The enhanced hexokinase, phosphoglucoisomerase and aldolase activities, as well as the unaltered phosphorylase activity, suggest that the increase in glycogen content observed by V. N. Singh, T. A. Venkitasubramanian & R. Viswanathan (unpublished work) is due to increased glycogen synthesis via the uridine diphosphate glucose-linked route without any alteration in its catabolic phase. It seems reasonable to assume here that increased hexokinase activity augments the phosphorylation of glucose and that the glucose 6-phosphate thus produced is channelled both towards the synthesis of glycogen, producing thereby an increase in the glycogen content, and towards the glycolytic pathway, thereby increasing the production of lactic acid in guinea-pig lung in experimental bagassosis.

SUMMARY

1. The activity of certain glycolytic enzymes, namely phosphorylase, hexokinase, phosphoglucoisomerase and aldolase, was estimated in lungs of guinea pigs which have been exposed to bagasse dust and was compared with that of normal guinea-pig lungs.

2. Phosphorylase activity was unaltered, whereas the activities of the other three enzymes were significantly increased in experimental bagassosis.

3. The lactic acid content was increased in the lungs of guinea pigs exposed to bagasse dust as compared with those of normal ones.

4. The significance of the results in explaining the increased glycogen content of guinea-pig lung in experimental bagassosis is discussed.

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Distribution of Unsaturated Fatty Acid in Pyridoxine-Deficient Hypercholesterolaemia

BY AJIT GOSWAMI* AND D. P. SADHU
Department of Physiology and Nutrition, Bengal Veterinary College, Calcutta 37, India

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The present study was prompted by the suggestion of Sinclair (1956) that the development of atherosclerosis may be related to a deficiency of unsaturated fatty acids. Since pyridoxine is known to be a factor for the conversion of linoleate into arachidonate (Witten & Holman, 1952), the atherosclerosis observed in pyridoxine-deficient monkeys (Rinehart & Greenberg, 1959) may be related to the deficiency of arachidonate. To test the validity of this hypothesis, a detailed study has been made of the unsaturated fatty acid content of the blood and arteries of pyridoxine-deficient rats.

Whether the hypercholesterolaemia observed was due to increased mobilization of lipids from liver or to some intrinsic metabolic aberration was tested by determining the cholesterol content of bile, which is known to reflect faithfully the status of cholesterol synthesis by the liver (Friedman & Byers, 1954).

* C.S.I.R. Senior Fellow.

EXPERIMENTAL

Induction of pyridoxine deficiency. Rats (60) of 60–85 g. body wt. were fed on a purified diet containing vitamin-free casein (22·0%), sucrose (66·5%), fat (coco-nut oil; 9·0%), salt mixture (2·5%) and sulphaguanidine for depletion of vitamins. After 3 weeks the animals were divided into three groups of 20 each. Animals of group 1 served as control and received the basal diet supplemented with a vitamin mixture containing (mg./kg. of diet) the following: calcium pantothenate (44); vitamin A concentrate (200 units/ml.; 99); α-tocopherol (225·5); inositol (301·4); menadione (4·4); biotin (0·7); niacin (595·5); *p*-aminobenzoic acid (22); riboflavin (22); pyridoxine hydrochloride (46); thiamine hydrochloride (22); choline chloride (601·0); folic acid (0·2 μg./kg.). Group 2 animals (inanition group) also received the same diet but it was adjusted in amount so that their weight gain corresponded to that of group 3 rats (deficient group), which received the same diet with the exception of pyridoxine hydrochloride. Body-weight gains were recorded daily and after 9 weeks the animals were killed. Blood was drawn directly from the heart after