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ON THE RELATIONSHIP BETWEEN METABOLIC ACTIVITY AND CHOLESTEROL UPTAKE BY INTIMA-MEDIA OF THE RABBIT AORTA

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SUMMARY

The relationship between metabolic activity and cholesterol uptake by intima-media of the rabbit aorta has been studied by use of a method *in vitro* previously described.

A highly significant, positive correlation was found between intima-media cholesterol uptake and metabolic activity as expressed by lactate and CO_2 production for control hemisegments showing the same percental variation in these two parameters. This relationship might also apply if cholesterol uptake was related only to the glycolytic pathway.

CN^- at a concentration of 0.5 mM provoked an increase in intima-media cholesterol uptake. It is therefore concluded that cholesterol uptake does not depend on energy from the oxidative pathway. Judged from the lactate production cyanide brought about an increase in glucose catabolism *via* the glycolytic pathway, even when the decrease in CO_2 production is taken into account.

Intima-media cholesterol uptake could be lowered in the presence of glycolytic inhibitors when lactate production by the inhibited aortic hemisegments was not too low. At a low lactate production by the inhibited hemisegment an increase in intima-media cholesterol uptake was seen, but a flabby appearance of the aortic tissue after incubation in such cases and a decrease in temperature dependence for cholesterol uptake supported the suggestion of a breakdown of the endothelial membrane barrier at low lactate production.

The extrapolated value for intima-media cholesterol uptake in controls at a lactate production of zero was equal to a value for a basic cholesterol uptake in experiments with F^- (5 mM), where this inhibitor no longer was able to bring about an inhibition in cholesterol uptake. This finding is in accordance with a previous finding of a non-temperature-dependent part of cholesterol uptake during a 4-h incubation, *viz.* the initial binding phase.

From previous observations on temperature dependence for the cholesterol transport across the intimal cell surface and from the results reported in this study it is concluded that the unidirectional transport of cholesterol from serum to intima-media under 'normal' conditions *in vitro*, where cholesterol molecules seem to be most impeded in their passage through the luminal endothelial cell membrane, is an energy-requiring process. Active glycolysis probably provides the immediate energy supply required. The results lend support to a previously stated suggestion of a pinocytotic

mechanism involved in the cholesterol transport across the endothelial cell membrane. It is emphasized, however, that only indirect proof exists for such a concept, for which a demonstration of labelled cholesterol in the vesicles of the endothelial cells is rather crucial.

INTRODUCTION

The endothelial cells of the arterial intima, as depicted by electron microscopists, contain numerous vesicles[1–4]. PALADE[1] in 1953 proposed that such endothelial vesicles might represent a transport system. The mechanism whereby cholesterol or lipoproteins are taken up at the endothelial cell surface has previously been the subject of studies based on that proposal. Thus it was found[5,6] that the kinetics of cholesterol uptake *in vitro* at the endothelial cell surface of the rabbit aorta were similar to those described for the uptake of proteins by amoeba[7] and ascites tumor cells[8], and of colloidal gold by macrophages[9], presumably due to pinocytosis. Furthermore, in various hyperlipemic conditions endothelial cells have been reported as being in the process of ingesting some electron-dense material, supposedly lipoprotein[10–12].

If the mechanism for serum cholesterol uptake at the endothelial cell surface is pinocytosis, it might be expected that variations in the metabolic activity of the cells involved would influence the rate of uptake. Thus pinocytotic uptake of proteins in amoeba was decreased by inhibitors interfering with oxidative metabolism[7,13,14] or with glycolysis[14]. A similar dependence on metabolic activity has been described for phagocytosis in leucocytes[15–18], although only glycolytic inhibitors were effective.

An individual variation in [^{14}C]cholesterol uptake by intima-media of the rabbit aorta during incubation in the same serum and at the same temperature has previously been described[19]. The present paper is concerned with an investigation of the possibility that these individual variations in [^{14}C]cholesterol uptake may be due to variations in metabolic activity. The influence of some metabolic inhibitors upon [^{14}C]cholesterol uptake will also be considered.

MATERIALS AND METHODS

White female rabbits from the same strain were obtained from the Danish State Serum Institute. They were used at an age of 4–6 months, and their weights ranged from 2.26 to 3.20 kg.

The preparation of aortic hemisegments, the serum labelling procedure, the incubation technique and the analytical details were as described previously[19]. The label used was ^{14}C in the biologically stable 4-position of the cholesterol molecule. Following incubation of the aortic hemisegments in serum containing [^{14}C]cholesterol, intima-media layers were prepared from the central parts of right and left hemisegments, cut out at two levels of the thoracic aorta. One of the two corresponding intima-media layers from the same level of the thoracic aorta served as a control for the other. The incubations were for 4 h at 38°. Lactic acid and pH were determined before and after incubation, the former by use of an enzymatic method[20–22], the latter at 38° using a direct-reading pH meter 22 (Radiometer, Copenhagen). Glucose disappearance was determined in some experiments with CN^- by an enzymatic method[23], using reagents from KABI, Stockholm, Sweden.

It is not possible to measure O_2 consumption by the direct Warburg technique[24], when CO_2-equilibrated serum is used as incubation medium. It is possible, however, to estimate the CO_2 production during incubation, if besides the above-measured parameters the CO_2 initially dissolved in serum, the gas phase in the closed system consisting of Warburg flasks and manometers[19], and the pH–$[HCO_3^-]$ equilibration curves[25] for rabbit serum are known, and it is assumed that the change in serum base excess can be ascribed only to the lactic acid production. The initial concentration of dissolved CO_2 in serum is fixed by the aeration of serum with a gas mixture moistened with water at 38° and of known composition of dry gases including CO_2. The size of the gas phase above the serum was not registered individually in these experiments, and so an average figure of 13.9 ml was used. This figure was calculated from previous investigations on O_2 consumption using the same apparatus equipment in 41 different combinations of manometers and Warburg flasks. The variation coefficient was 4 %.

The pH–$[HCO_3^-]$equilibration curves were obtained by use of an Astrup microtonometer[26]. Equilibration took place at 38° using three different but known CO_2 tensions. An average value for the slope of the pH–$[HCO_3^-]$equilibration curves from four different rabbit sera was used in the calculation of CO_2 production (-9.73 ± 0.88 mM per pH unit).

Net lactate production (henceforth called only lactate production) and CO_2 production are determined for the whole aortic hemisegment, whereas [^{14}C]cholesterol uptake is measured later on an isolated intima-media preparation of the central part of the incubated hemisegment. In order to establish a possible relationship between metabolic activity of intima-media and of intima-media and outer layer, aortic hemisegments were divided into intima-media and outer layer, each of which was incubated for 4 h at 38° in a modified Krebs–Ringer–Tris solution with 0.2 % glucose for the measurement of lactate production and O_2 consumption, according to the procedure described previously[19]. Among 16 incubations NaF and NaCN were added to the incubation medium, each in 4 experiments, in order to obtain a great variation in the metabolic parameters.

In the [^{14}C]cholesterol uptake experiments the following metabolic inhibitors were used: (1) NaCN, (2) 2-deoxyglucose, (3) iodoacetic acid and (4) NaF. These substances were added to the serum containing [^{14}C]cholesterol in a volume not exceeding 1 % of the serum volume. To the controls (1) NaCl, (2) glucose, (3) acetic acid or HCl and (4) NaCl were added to obtain the same concentration as that of the inhibitor used. Model experiments showed that CaF_2 was precipitated in concentrations of F^- higher than 5 mM.

Calculation of CO_2 production

Symbols used: $[HCO_3^-]^\circ$ and $[HCO_3^-]^{4h}$: initial and final concentration of serum HCO_3^- (mM). $[CO_2]^\circ$ and $[CO_2]^{4h}$: initial and final concentration of dissolved CO_2 in serum (mM). pH° and pH^{4h}: initial and final serum pH at 38°. V: volume of gas phase above serum (at 38° saturated with water vapour), 13.9 ml. $CO_2{}^S$: amount of dissolved CO_2 in serum (ml). $CO_2{}^T$: $CO_2{}^S$ *plus* amount of CO_2 in gas phase above serum (ml). α_{311}: absorption coefficient for CO_2 in serum at 311 °K, 0.51.

$$a: \frac{CO_2{}^T}{CO_2{}^S} = \frac{V \cdot 273}{311 \cdot 2 \cdot \alpha_{311}} + 1 = 13.0 \text{ (variation coefficient, 3.5 \%)}$$

b: slope of pH-$[HCO_3^-]$ equilibration curve for rabbit serum, —9.73 mM per pH unit. F_{CO_2}: fraction of CO_2 in the gas used for aeration of serum before incubation, 0.058. Δ[lactate]: change in serum lactate concentration (mM). $Q_{CO_2}{}^{4h}$: amount of CO_2 produced per 4 h (μmoles/4 h).

The amount of serum used in each incubation is 2 ml. $Q_{CO_2}{}^{4h}$ can now be calculated from the following equation:

$$Q_{CO_2}{}^{4h} = 2[[HCO_3^-]^{4h} - [HCO_3^-]^\circ + a([CO_2]^{4h} - [CO_2]^\circ)] \quad (1)$$

As the pH-$[HCO_3^-]$ equilibration curves can be described as straight, parallel lines within the small pH interval[25] and assuming that the change in base excess during incubation equals Δ[lactate], we obtain the following equation:

$$[HCO_3^-]^{4h} = [HCO_3^-]^\circ + b(pH^{4h} - pH^\circ) - \Delta[\text{lactate}] \quad (2)$$

and from the Henderson–Hasselbalch equation:

$$[CO_2]^{4h} = 10^{6.1 - pH^{4h}} \cdot [HCO_3^-]^{4h} \quad (3)$$

Combining Eqns. 1, 2 and 3 and inserting the values for the common constants used (a and b), we get:

$$Q_{CO_2}{}^{4h} = 2[-9.73(pH^{4h} - pH^\circ) - \Delta[\text{lactate}] + 13.0[10^{6.1 - pH^{4h}}([HCO_3^-]^\circ - 9.73(pH^{4h} - pH^\circ) - \Delta[\text{lactate}]) - [CO_2]^\circ]]$$

in which $[CO_2]^\circ = F_{CO_2}(B - 50) \cdot 0.030$, where B is the barometric pressure and 50 is the pressure of saturated water vapour at 38° in mm Hg, and

$$[HCO_3^-]^\circ = 10^{pH^\circ - 6.1} \cdot [CO_2]^\circ$$

Measuring pH°, pH^{4h} and Δ[lactate], it is now possible to determine the CO_2 production.

RESULTS

Fig. 1 shows the relationship between lactate production by intima-media and by intima-media *plus* outer layer, and between O_2 consumption by intima-media and by intima-media *plus* outer layer, both within the same aortic hemisegment. In both cases the correlation coefficient is 0.94, ($P < 0.001$), thus indicating a highly significant, positive correlation between the whole incubated hemisegment and its intima-media layer as regards two of the main metabolic parameters.

In Fig. 2 is shown the relationship between CO_2 production and lactate production by aortic hemisegments incubated in the same labelled serum in which the individual variations of these two metabolic parameters were great and percentally equal (*cf.* that the correlation coefficient for the rectilinear regression is 0.95 ($P < 0.001$) and that the last term of the equation shown in the figure (0.37) does not significantly differ from zero). Thus, CO_2 production $= k_1 \cdot$(lactate production). The metabolic activity expressed by lactate and CO_2 production is

$k_2 \cdot$(lactate production) + $k_3 \cdot$(CO_2 production).

Substituting k_1·(lactate production) for CO_2 production, we obtain the following equation:

$$\text{Metabolic activity} = (k_2 + k_1k_3)\ (\text{lactate production})$$

The metabolic activity of these aortic hemisegments may therefore be assumed to vary individually as either of the two metabolic parameters.

Fig. 3 shows the relationship between intima-media cholesterol uptake and metabolic activity by the corresponding whole aortic hemisegment for the samples of Fig. 2. As k_2 and k_3 are unknown the metabolic activity is expressed in arbitrary units per mg per 4 h. The regression line shown in the figure has the following equation: $y = 0.568x + 0.042$. The regression coefficient is significantly different from zero ($P<0.005$), and the correlation coefficient is 0.89. The last term of the equation, which may be interpreted as the cholesterol uptake at a metabolic activity of zero, has a value of 0.042 ± 0.019 $\mu g/cm^2$ and is thus barely significantly different from zero at a 5 % level.

The effect of various metabolic inhibitors on intima-media cholesterol uptake

CN^-. The effect of CN^- on intima-media cholesterol uptake was investigated at a concentration of 0.5 mM using two different sera (A and B) as incubation media. The cholesterol concentration of serum A and B was 40.3 and 48.7 mg per 100 ml, respectively. Lactate production, CO_2 production and glucose disappearance were determined in the experiments, where serum A was used as incubation medium. In calculation of the overall effect of CN^- on the 4-h uptake of cholesterol the uptake was converted at the same cholesterol concentration, *viz.* 45 mg per 100 ml, by use of a formula previously described[5]. The correction factor is maximally 1.07. The results are summarized in Table I.

It appears that CN^- (0.5 mM) brought about a 49% inhibition of CO_2 production, a 161 % increase in lactate production and a 81 % increase in glucose disappearance, when intima-media *plus* outer layer is considered. From the observations shown in Figs. 1a and 1b it is reasonable to assume similar changes at least in lactate and CO_2 production by intima-media. As to the effect of 0.5 mM CN^- on cholesterol

TABLE I

THE EFFECT OF CN^- ON INTIMA-MEDIA CHOLESTEROL UPTAKE AND LACTATE PRODUCTION, CO_2 PRODUCTION AND GLUCOSE DISAPPEARANCE FOR SOME OF THE CORRESPONDING WHOLE AORTIC HEMISEGMENTS DURING A 4-h INCUBATION IN SERUM CONTAINING [^{14}C]CHOLESTEROL

The figures are mean values of the number of samples indicated in parentheses, in the last column ± S.E. For further information, see text.

Serum	*Condition*	*Lactate production (μmoles/mg per 4 h)*	*CO_2 production (μmoles/mg per 4 h)*	*Glucose disappearance (μmoles/mg per 4 h)*	*Cholesterol uptake ($\mu g/cm^2$ per 4 h)*	*Change in cholesterol uptake due to CN^- ($\mu g/cm^2$ per 4 h)*
A	Controls (6)	0.098	0.270	0.083	0.079	$+0.033 \pm 0.015$
	0.5 mM CN^- (6)	0.256	0.137	0.150	0.112	$0.05<P<0.1$
A + B	Controls (9)	—	—	—	—	$+0.030 \pm 0.011$
	0.5 mM CN^- (9)	—	—	—	—	$P<0.05$

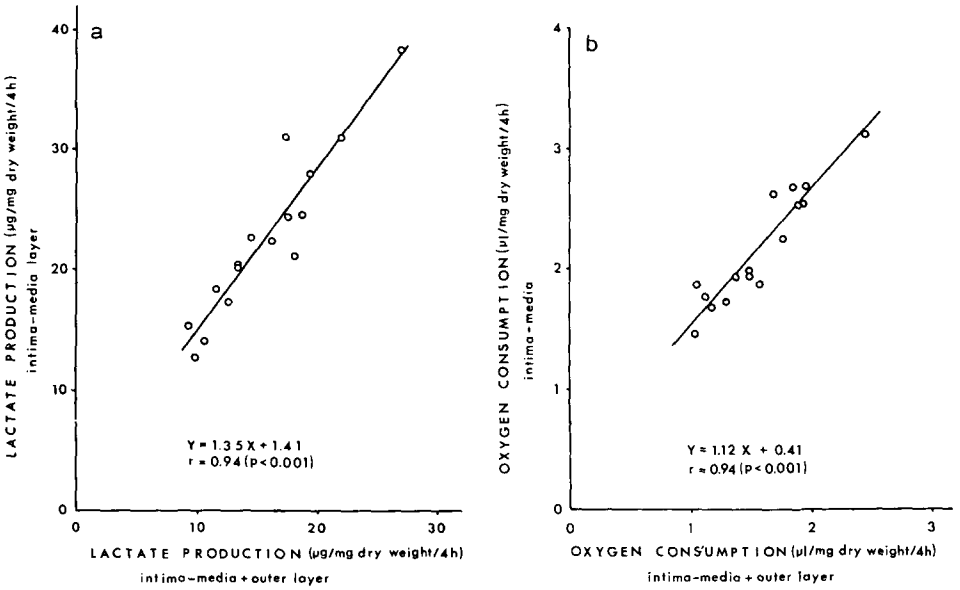

Fig. 1. a. The relationship between net lactate production by intima-media and the corresponding whole aortic hemisegment (intima-media *plus* outer layer) during a 4-h incubation in a modified Krebs–Ringer–Tris solution with 0.2% glucose. b. The relationship between O_2 consumption by intima-media and the corresponding whole aortic hemisegment (intima-media *plus* outer layer) under the same experimental conditions as in (a). The regression lines and correlation coefficients are shown in the figures.

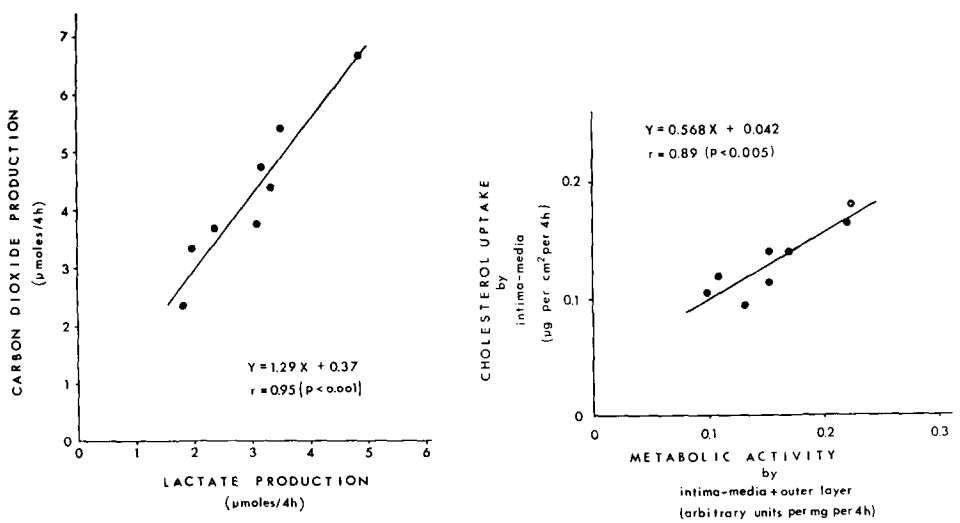

Fig. 2. The relationship between CO_2 and net lactate production by control aortic hemisegments during a 4-h incubation in the same [^{14}C]cholesterol-containing serum. The regression line and correlation coefficient are shown in the figure.

Fig. 3. The relationship between intima-media cholesterol uptake and metabolic activity by the same whole aortic hemisegments as in Fig. 2. The regression line and correlation coefficient are shown in the figure.

uptake by intima-media, an overall average increase of 0.030 ± 0.011 μg per cm^2 per 4 h was observed ($P<0.05$).

2-Deoxyglucose and iodoacetate. In the experiments with these two glycolytic inhibitors two different sera (I and II) were used, but the concentration of serum cholesterol was almost equal (48.1 and 49.6 mg per 100 ml). The effect of 2-deoxyglucose was investigated at 2 concentrations of the inhibitor, *viz.* 5 and 10 mM, and the effect of iodoacetate was studied at a concentration of 0.1 mM. The results are shown in Table II.

TABLE II

THE EFFECT OF 2-DEOXYGLUCOSE AND OF IODOACETATE ON INTIMA-MEDIA CHOLESTEROL UPTAKE AND LACTATE PRODUCTION BY THE CORRESPONDING WHOLE AORTIC HEMISEGMENTS DURING A 4-h INCUBATION IN SERUM CONTAINING [^{14}C]CHOLESTEROL

The figures are mean values of the number of samples indicated in parentheses, in the last column ± S.E.

Serum	*Condition*	*Lactate production (μmoles/mg per 4 h)*	*Cholesterol uptake (μg/cm² per 4 h)*	*Change in cholesterol uptake (%)*
I	Controls (6)	0.099	0.096	−17.2 ± 6.2
	5 mM 2-deoxyglucose (6)	0.086	0.079	$P<0.05$
	Controls (6)	0.083	0.081	+18.2 ± 12.4
	10 mM 2-deoxyglucose (6)	0.058	0.093	Not significant
II	Controls (6)	0.150	0.135	+ 6.7 ± 5.0
	10 mM 2-deoxyglucose (6)	0.073	0.143	Not significant
	Controls (5)	0.149	0.157	+73.1 ± 23.0
	0.1 mM iodoacetate (5)	0.038	0.254	$P<0.05$

It appears from Table II that the lactate production was greater for the aortic hemisegments of the controls incubated in serum II than for those incubated in serum I. According to the observations shown in Fig. 1a the same presumably holds true for lactate production by intima-media. Corresponding to this a higher intima-media cholesterol uptake is found for the controls incubated in serum II than for those incubated in serum I. Furthermore, it appears that the effect of glycolytic inhibition on cholesterol uptake may be dual. At a slight inhibition of lactate production (13 % in the experiments with 5 mM 2-deoxyglucose) a significant decrease in cholesterol uptake is seen. At a strong inhibition of lactate production (74 % in the experiments with 0.1 mM iodoacetate) a significant increase in cholesterol uptake is observed. The aortic hemisegments incubated in serum with iodoacetate did, however, present a flabby appearance, suggesting a tissue-damaging effect. This could not be observed in the other experiments. This finding led to an investigation of the effect of temperature upon [^{14}C]cholesterol uptake by intima-media layers from hemisegments preincubated for 15 min in a Krebs–Ringer solution with 0.2 % glucose and iodoacetate at a concentration of 5 mM. After preincubation corresponding aortic hemisegments were incubated for 4 h in labelled serum without iodoacetate, at 38 and 4°, respectively. The ratio between [^{14}C]cholesterol uptake at 4 and 38° was, in five experiments, found to be: 0.12, 0.18, 0.45, 0.85 and 0.95. This ratio was previously found to be 0.17 with a standard deviation between 0.06 and 0.07 in experiments

without iodoacetate[5]. Thus in three out of five experiments, preincubation with 5 mM iodoacetate led to a decrease in the effect of temperature upon [^{14}C]cholesterol uptake.

F^-. In a group of six control incubations and six incubations with 5 mM F^- the average lactate production during a 4-h incubation was 0.152 and 0.097 μmole/mg, respectively. The cholesterol concentration of the serum used for incubation was 64.3 mg per 100 ml, and the specific activity of serum cholesterol was 209 counts/min per μg. The effect of F^- on the 4-h uptake of cholesterol was an average percental decrease of 24.4% (Fig. 5), but the large spontaneous, individual variation in cholesterol uptake by the control intima-media layers in these experiments made possible a further analysis of the inhibition at various control uptake values (Fig. 4).

It appears from Fig. 4 that only when the cholesterol uptake by the control intima-media was above a certain value (the intercept value on the abscissa) did F^- decrease the uptake. The percentual inhibition of the cholesterol uptake above this value is 100 times the slope of the rectilinear regression line shown in the figure, *i.e.*, 76 ± 4% ($P<0.001$). The basic value for cholesterol uptake, where F^- is no longer able to inhibit cholesterol uptake, is 0.047 μg per cm^2 per 4 h.

Cholesterol uptake as a function of lactate production by the inhibited aortic hemisegments

Fig. 5 shows the cholesterol uptake by intima-media layers exposed to the above glycolytic inhibitors as a function of lactate production by the corresponding aortic hemisegments. The uptake is expressed relative to that of the corresponding intima-

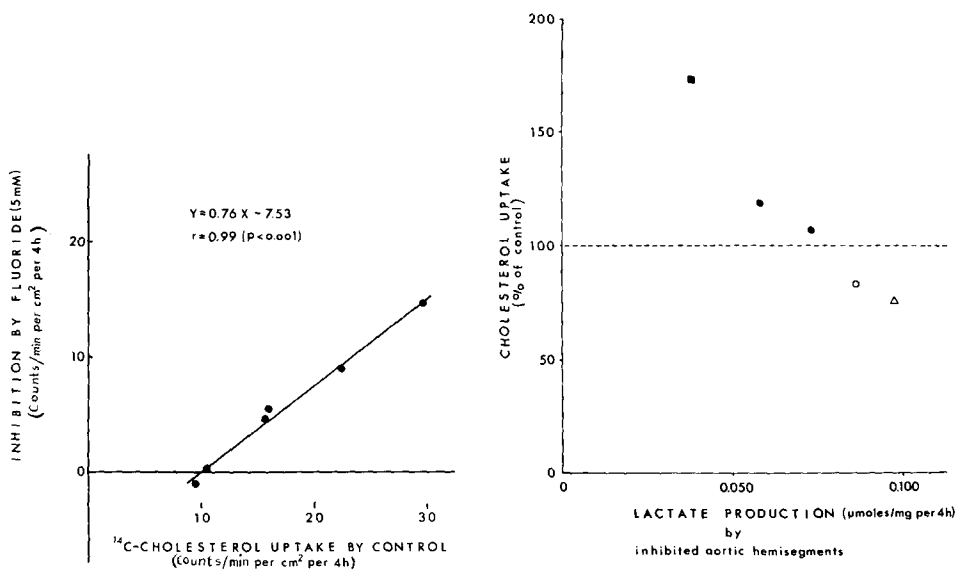

Fig. 4. The relationship between absolute inhibition of intima-media cholesterol uptake caused by F^- (5 mM) and cholesterol uptake by the corresponding intima-media control during a 4-h incubation. The regression line and correlation coefficient are shown in the figure. For further explanation see text.

Fig. 5. The relative uptake of cholesterol by intima-media layers as a function of net lactate production by their corresponding aortic hemisegments exposed to various glycolytic inhibitors (■, 0.1 mM iodoacetate; ●, 10 mM 2-deoxyglucose; ○, 5 mM 2-deoxyglucose; and △, 5 mM F^-). The points are mean values, each calculated from 5–6 paired observations.

media. It appears from the figure that there is a continuous transition from decrease to increase in cholesterol uptake at decreasing lactate production.

DISCUSSION

The experimental procedure used for the measurement of [^{14}C]cholesterol uptake at the endothelial cell surface includes the isolation of an intima-media layer from the central part of the incubated aortic hemisegment[19]. The lactate and CO_2 production observed during the incubations are on the other hand due to the metabolism of the whole aortic hemisegment incubated. The cholesterol uptake by intima-media can therefore not be correlated primarily with the metabolic activity of intima-media. It appears from Figs. 1a and 1b, however, that a highly significant, positive correlation exists as to lactate production and O_2 consumption between intima-media and the corresponding whole aortic hemisegment (intima-media *plus* outer layer), and so it is assumed that the same holds true for lactate and CO_2 production in the cholesterol-uptake experiments. This implies that the quantitative relationship between cholesterol uptake and lactate and CO_2 production by intima-media may be described by an equation similar to that found for the regression line in Fig. 3, only with a change of the slope.

The metabolic activity is in this study expressed as

$$k_2 \text{ (lactate production)} + k_3 \text{ (CO_2 production)}.$$

As k_2 and k_3 are unknown, the metabolic activity is given in arbitrary units, and a comparison of different metabolic activities can only be made, when the lactate and CO_2 production change with the same factor, which is the case for the aortic hemisegments used in Fig. 2. In addition the CO_2 production is determined very indirectly, which means that greater importance should be attached to percentual changes in CO_2 production than to absolute values of this parameter.

The cholesterol uptake at a theoretical metabolic activity of zero according to Fig. 3 is 0.042 μg/cm^2 at a total cholesterol concentration of 48.1 mg per 100 ml The cholesterol uptake, which according to Fig. 4 cannot be inhibited by F^-, is in these experiments 0.047 μg/cm^2 at a total cholesterol concentration of 64.3 mg per 100 ml or converted at a total cholesterol concentration of 48.1 mg per 100 ml, 0.040 μg/cm^2. Probably these figures of equal size correspond to the initial uptake —the amount bound to the endothelial cell surface in the above experiments—which in a previous study[5] was found to be independent of temperature and hence also thought to be independent of metabolic activity.

Intima-media cholesterol uptake was increased in the presence of CN^- at a concentration of 0.5 mM. This observation seems to indicate that intima-media cholesterol uptake does not depend on energy from the oxidative pathway. Such a statement is not inconsistent with the finding in Fig. 3 of a rectilinear relationship between cholesterol uptake and the expression used for metabolic activity:

$$k_2 \text{ (lactate production)} + k_3 \text{ (CO_2 production)}$$

in which CO_2 production $= k_1 \cdot$(lactate production). This relationship might also apply if the cholesterol uptake were related only to the Embden–Meyerhof pathway. The increase in lactate production in the presence of CN^- points to a higher rate in

the Embden–Meyerhof pathway, even when the decrease in CO_2 production is taken into account. Corresponding to this the glucose disappearance is greater in the experiments with CN^- than in the control experiments. The increase in intima-media cholesterol uptake in the presence of CN^- may thus be due to a stimulation of the glycolytic pathway.

In the presence of glycolytic inhibitors intima-media cholesterol uptake was decreased when the lactate production by the inhibited aortic hemisegments was not too low. When the glycolysis was strongly inhibited, as in the experiments with iodoacetate, the flabby appearance of the aortic tissue together with the finding of a tendency toward decreasing temperature dependence for the 4-h uptake of cholesterol after 15 min of preincubation with 5 mM iodoacetate might indicate that the increase in cholesterol uptake was due to a breakdown of a membrane barrier[27]. Such an effect seems to occur gradually at decreasing lactate production by the inhibited aortic tissue (Fig. 5). An hypothesis about a metabolic barrier to the influx of cholesterol was previously proposed by SHAPIRA *et al.*[28] and by ZILVERSMIT AND NEWMAN[29].

From previous investigations on the effect of temperature upon intima-media cholesterol uptake[5] and from the observations reported in this study, the conclusion is reached that the initial uptake representing the binding phase does not depend on temperature or metabolic activity, whereas the unidirectional transport from serum to intima-media depends on temperature and metabolic activity related to the glycolytic pathway. The dependence upon the latter parameter seems to occur in two different ways. When the endothelial barrier is thought to be intact, intima-media cholesterol uptake can be lowered by inhibition of glycolysis and increased in the presence of CN^-, which stimulates glycolysis, according to the observation that individual variations in cholesterol uptake by control intima-media layers are parallel to percentally equal variations in lactate and CO_2 production. When the lactate production of the aortic hemisegments becomes too low due to inhibition of glycolysis, the endothelial barrier is broken down, resulting in an increased 'permeability' for cholesterol. The dependence of intima-media cholesterol uptake on metabolic activity related to the glycolytic pathway under 'normal' conditions *in vitro*, where cholesterol molecules seem to be most impeded in passage through the endothelial cell membrane, lends further support to an earlier statement that a pinocytotic mechanism is involved in cholesterol transport across the intimal surface from the luminal side. It should be emphasized, however, that no direct proof exists for this concept, for which a demonstration of labelled cholesterol in the vesicles of the endothelial cells is rather crucial.

With the assumption of a metabolic barrier to cholesterol in the arterial wall several observations make sense (*e.g.*, reviewed by CONSTANTINIDES[30]): mechanical injury, chemical poisons or anoxia might accelerate cholesterol accumulation in the arterial wall during hyperlipemia by affecting enzyme systems involved in maintaining the endothelial barrier.

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