Br. J. Pharmac. (1973), **47**, 305–314.

Inhibition of functional vasodilatation and prostaglandin formation in rabbit adipose tissue by indomethacin and aspirin

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Summary

1. The epigastric adipose depot of rabbits has been used to examine the effect of indomethacin, aspirin and nicotinic acid on the free fatty acid release and blood flow in fat tissue.

2. The prostaglandin formation which occurs in adipose tissue during lipolysis is prevented by indomethacin and aspirin. The corresponding functional vasodilatation which occurs after infusions of lipolytic substances is also abolished by these two anti-inflammatory compounds.

3. This finding is consistent with the theory that prostaglandin E_2 is the mediator of functional vasodilatation in adipose tissue.

4. Nicotinic acid sometimes inhibits the release of free fatty acids from adipose tissue by preventing activation of the tissue lipase. In those experiments in which this inhibition occurred, the vasodilatation was also prevented.

5. This finding is consistent with the view that the prostaglandin E_2 which mediates the functional vasodilatation, originates in the triglycerides of the fat tissue.

Introduction

Active lipolysis in adipose tissue is accompanied by an increased blood flow through the tissue. Ngai, Rosell & Wallenberg (1966) found that although during a period of sympathetic nerve stimulation of dog subcutaneous adipose tissue the blood vessels were constricted, after the stimulation there was a release of free fatty acids which was sometimes accompanied by a vasodilatation. Nielsen, Lbitsch, Larsen, Lassen & Quaade (1968) showed that intravenous infusion of noradrenaline, or injection of glucagon, or fasting, i.e. procedures which have a pronounced lipolytic effect in man, also cause an increase in blood flow in adipose tissue. In rabbits, a species in which adipose tissue is relatively insensitive to catecholamines (Rudman, Brown & Malkin, 1963; Boberg, Micheli & Rammer, 1970), Lewis & Matthews (1970) showed not only that the blood flow through the tissue was increased during lipolysis but also that it was accompanied by the release or formation of a vasodilator substance in the tissue. Bowery, Lewis & Matthews (1970) subsequently identified the vasodilator substance as prostaglandin E_2. Vane (1971), Smith & Willis (1971) and Ferreira, Moncada & Vane (1971) have since shown that the anti-inflammatory compounds indomethacin and aspirin inhibit the formation of prostaglandin.

In the present investigation indomethacin and aspirin have been used to test the theory that prostaglandin E_2 is the mediator of functional vasodilatation in rabbit adipose tissue. In addition nicotinic acid has been used to learn more of the mechanism by which the prostaglandin is formed.

Methods

Infusion experiments

Adrenocorticotrophic hormone $(ACTH)^{1-24}$ and anti-inflammatory compounds were infused close arterially to the epigastric adipose tissue as described earlier by Lewis & Matthews (1970).

Extraction of adipose tissue

The method of Samuelsson (1963) as used by Bowery *et al.* (1970) was employed to extract prostaglandin-like activity from the fat tissue.

Biological assay

Samples were assayed either on the guinea-pig ileum or rat stomach strip suspended in oxygenated Tyrode solution at 34° C or 37° C respectively.

Extraction of free fatty acids

Blood samples were collected from the epigastric vein into glass tubes containing a little solid heparin and kept on ice until required. Free fatty acids were estimated colorimetrically by Duncombe's (1964) method on 0·25 ml plasma, with 3·0 ml copper reagent and 10 ml chloroform.

Materials

The following substances were used. Adrenocorticotrophic hormone $(ACTH)^{\beta-1-24}$ (Synacthen CIBA-GEIGY); indomethacin (Merck, Sharpe & Dohme); nicotinic acid (Hopkin & Williams).

Results

Blood flow experiments

Previously we showed that infusion of $ACTH^{1-24}$ close arterially into the epigastric fat depot caused not only an output of free fatty acids but also a prolonged vasodilatation. The vasodilatation following a 5 min infusion of $ACTH^{1-24}$ is illustrated in Fig. 1, which shows that the venous outflow from the adipose tissue is increased for as long as 20–25 minutes. This experiment was designed to show that several such infusions could be made without causing desensitization of the tissue. Figure 1B shows that a second infusion given 1 h after the first, produced about the same degree of vasodilatation which was maintained for as long as that after the first infusion. Figure 1C shows the vasodilatation produced by a fourth infusion of $ACTH^{1-24}$, each given at 1 hourly intervals; the response to the third infusion is not illustrated.

When indomethacin was infused into the fat depot immediately before the $ACTH^{1-24}$ the vasodilator response was considerably reduced. After a 3–5 min

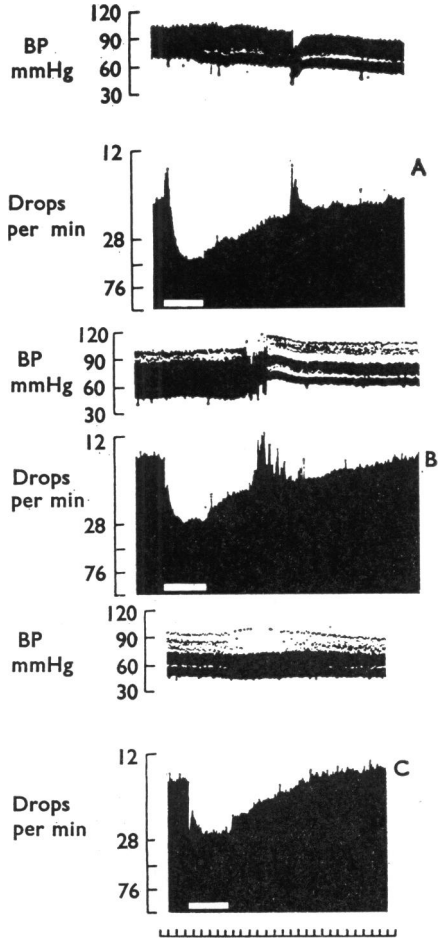

FIG. 1. Rabbit, male, 3·5 kg. In each record A, B and C, the upper record is of arterial blood pressure, the lower record of venous outflow from the epigastric adipose tissue, in drops/minute. The latter was measured with a Gaddum drop counter. At the white bars adrenocorticotrophic hormone 1 μg/min was infused close arterially to the epigastric fat pad. Time marks, 1 min; between A and B 1 hour; between B and C 2 hours. An additional infusion was made between B and C which is not shown.

TABLE 1. *Effects of indomethacin and aspirin on vasodilatation produced by infusion of different amounts of adrenocorticotrophic hormone$^{\beta-1-24}$ (ACTH)*

Experiment	Total dose of inhibitor	Infusion time (min)	Dose ACTH (μg/min)	% Inhibition vasodilatation
	Indomethacin			
1	20 μg	20	1	79
2	50 μg	5	1	58
	30 μg	3	1	59
3	100 μg	10	1	88
	200 μg	10	1	92
4	200 μg	10	0·3	87
5	1·5 mg	15	0·15	74
	1·5 mg	15	0·15	98
	Aspirin			
6	0·5 mg	10	0·3	35
7	10 mg	10	1	30
	10 mg	10	1	68
8	30 mg	10	1	55
	30 mg	30	1	100

All infusions were made close arterially to the epigastric fat depot.

infusion of indomethacin 10–20 μg/min the vasodilator response to ACTH was reduced to about 40% of the control response while after a 10 min infusion the response was reduced to 10–15%. Table 1 shows the effect of infusions of varying amounts of indomethacin on the vasodilatation produced by the infusion of different amounts of $ACTH^{1-24}$. The table also shows the effect of infusions of aspirin.

The experiment of Fig. 2 is a typical one illustrating the normal vasodilator response to $ACTH^{1-24}$ in A and the response to a similar infusion given after a 10 min infusion of indomethacin 20 μg/min in B. Partial recovery of the response usually occurred 1–2 h after the infusion of indomethacin and this is illustrated in Fig. 2C where the infusion of $ACTH^{1-24}$ was given 90 min after the indomethacin. In these experiments the blood flow was increased during the infusion of indomethacin or aspirin but returned to normal soon after the infusion was stopped. A typical effect is shown for indomethacin in Figure 2B.

The inhibition of the vasodilator response to $ACTH^{1-24}$ by indomethacin was not due to antagonism of prostaglandin itself since it was possible to show that the vasodilatation following infusion of prostaglandin E_1 was not affected at a time when the vasodilatation produced by $ACTH^{1-24}$ was greatly reduced. The experiment shown in Fig. 3 illustrates this point. In Figure 3B the response to prostaglandin E_1 50 ng/min infused for 3 min was unaffected by indomethacin 100 μg/min

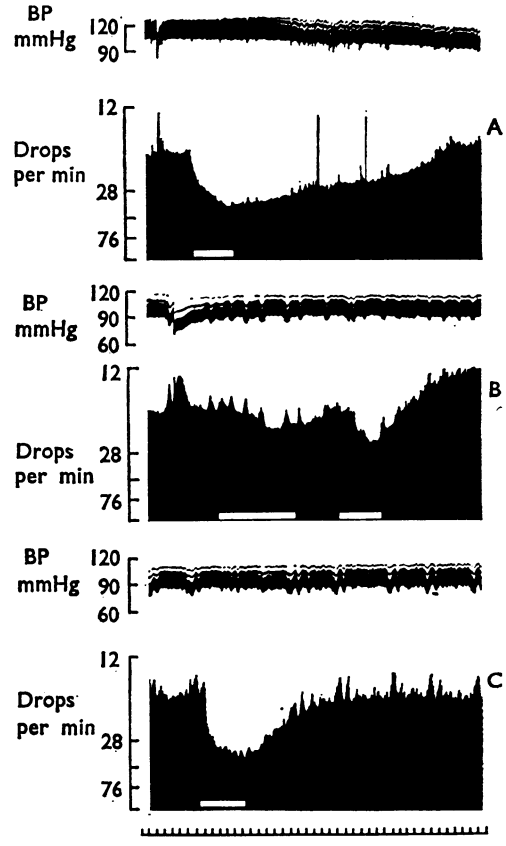

FIG. 2. Rabbit, male, 4 kg. Description of the record as for Figure 1. In B indomethacin 20 μg/min was infused during the period marked by the first white bar. Between A and B 2 hours ; and B and C 1·5 hours.

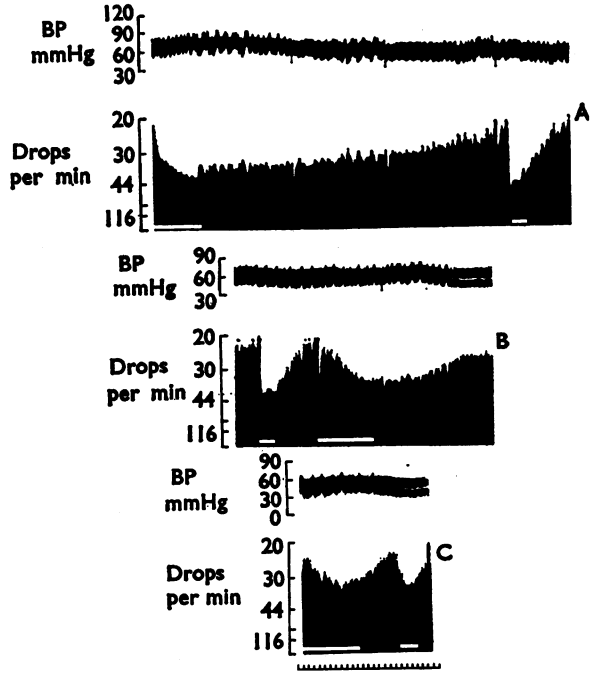

FIG. 3. Rabbit, female, 4 kg. Description of the record as for Figure 1. The white bars indicate close arterial infusions of adrenocorticotrophic hormone (ACTH) 150 ng/min and prostaglandin E_1 50 ng/min in A and C and prostaglandin E_1 50 ng/min followed by ACTH 150 ng/min in B. Between A and B, and B and C indomethacin 100 μg/min was infused close arterially to the fat pad for 15 minutes.

for 15 min (infused between 3A and 3B), whereas the response to $ACTH^{1-24}$ 150 ng/min was reduced to about 25% of that obtained before the indomethacin. The responses in Fig. 3C were obtained after a further infusion of indomethacin 100 μg/minute. After this additional indomethacin not only was the response to $ACTH^{1-24}$ further reduced but that to prostaglandin E_1 was reduced as well. This effect was probably due to non-specific inhibition of the tissue by the indomethacin, since at the time of Fig. 3C, although not illustrated, the vasodilator response to bradykinin was also significantly reduced.

Extract of fat pads

Previously we had found that alcohol and acid ether extracts of epigastric adipose tissue excised during a period of fat mobilization contained a substance which resembled prostaglandin E_2 and which contracted the isolated guinea-pig ileum. In the present experiments it has been shown that infusion of indomethacin immediately before activation of the fat depot prevented the appearance of prostaglandin in extracts of the fat pad. In the experiment shown in Fig. 4 the right epigastric fat depot (extract 1 in Fig. 4) was excised after a 15 min infusion of $ACTH^{1-24}$ 1 μg/minute. Subsequently indomethacin 100 μg/ml followed by $ACTH^{1-24}$ 1 μg/min were each infused for 15 min into the left epigastric fat depot of the same animal (extract 2 in Fig. 4) after which this fat pad too, was excised. Both fat pads were extracted into alcohol and then into acid ether and the residue taken up in Tyrode solution and assayed on the isolated guinea-pig ileum against

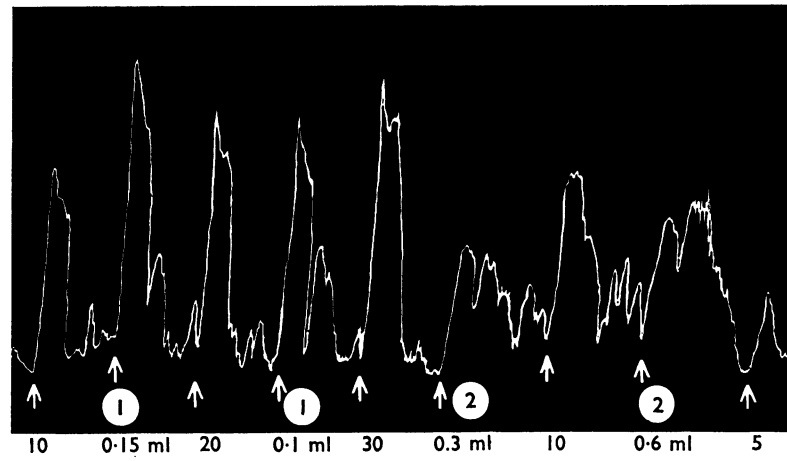

FIG. 4. Responses of guinea-pig ileum suspended in 5 ml Tyrode solution at 34° C, to prostaglandin E_1 in ng, and to extracts of adipose tissue, in ml. Extract one made from a fat pad excised during activation following infusion of adrenocorticotrophic hormone (ACTH) 1 μg/min for 15 minutes. Extract two made from a fat pad which had received an infusion of indomethacin 100 μg/ml for 15 min immediately before the infusion of ACTH.

prostaglandin E_1 as standard. Extract 1 contained 200 ng/ml while extract 2 contained less than 20 ng/ml of prostaglandin E_1 equivalent.

In 5 experiments in which indomethacin was infused before ACTH the mean values for prostaglandin E were 2·8 ng/g in the control tissue and $4{\cdot}82 \pm 1{\cdot}5$ ng/g in the stimulated tissue. These values indicate that not only had indomethacin reduced the prostaglandin formation on activation of the fat pad but had also reduced the resting level. In earlier experiments (Bowery *et al.*, 1970) the mean values for prostaglandin E were 17 ± 6 in the control tissue and 84 ± 24 after the tissue had been stimulated with ACTH.

Free fatty acid release

As shown in an earlier paper the free fatty acid concentration in the venous blood rose during an infusion of $ACTH^{1-24}$ reaching a maximum at the end of the infusion or shortly afterwards. In the present experiments it has been possible to show that although indomethacin inhibits prostaglandin formation and functional vasodilatation in the gland, lipolysis itself as measured by the output of free fatty acids was unaffected. In the experiment of Fig. 5 indomethacin 100 μg/ml was infused for 15 min close arterially into the fat pad immediately before a 15 min infusion of $ACTH^{1-24}$ 1 μg/minute. The free fatty acid concentration of the venous blood just after the infusion of $ACTH^{1-24}$ reached a level of about 4 mEq/l. and was therefore within the range of values found in the previous study in which the fat depots were activated by $ACTH^{1-24}$ without infusion of indomethacin (Lewis & Matthews, 1970). In two other such experiments a similar result was obtained.

In all three of these experiments the infusion of 100 μg/ml indomethacin itself caused a transient increase in the venous plasma concentration of free fatty acid which, however, returned to the resting level when the infusion was stopped as seen from Figure 5.

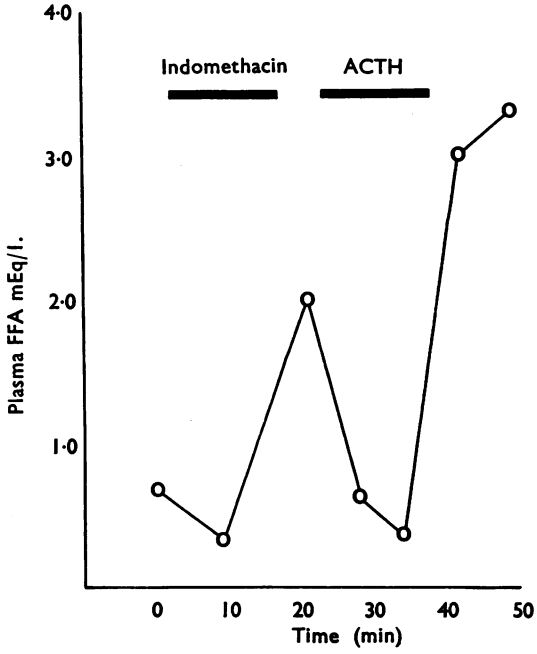

FIG. 5. Effect of close arterial infusions of indomethacin 100 μg/ml and adrenocorticotrophic hormone (ACTH) 1 μg/min to the epigastric fat depot on the venous free fatty acid (FFA) concentration.

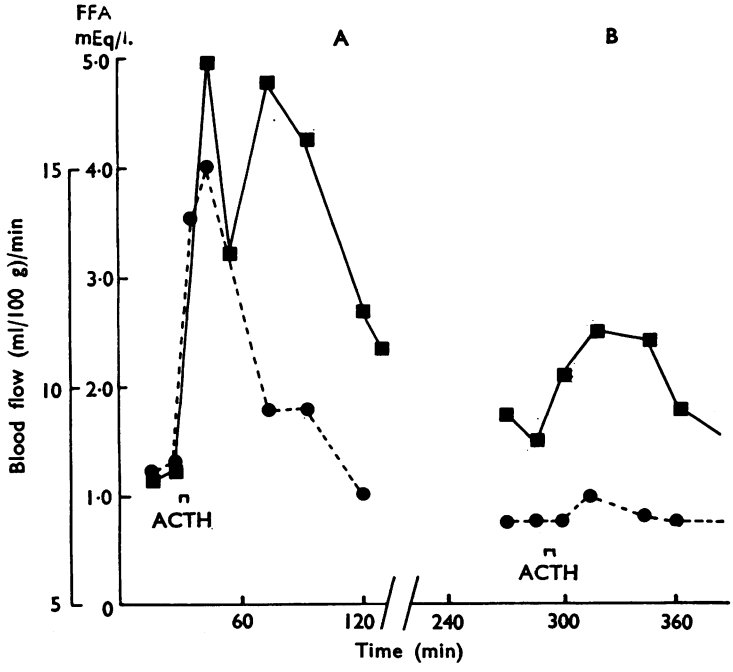

FIG. 6. Effect of close arterial infusions of adrenocorticotrophic hormone (ACTH) 1 μg/min to the epigastric fat depot before (in A) and after (in B) infusion of nicotinic acid 3 mg/min for 15 min on venous free fatty acid (FFA) concentration (■——■) in mEq/l. and venous outflow (●– –●) in ml/minute.

Inhibition of free fatty acid release

Nicotinic acid lowers the concentration of free fatty acids in plasma and prevents the increase in their concentration following injection of noradrenaline (Carlson & Orö, 1962). In the present investigation 4 experiments were carried out to examine the effect of a close arterial infusion of nicotinic acid 3 mg/min for 15 min on the ACTH-induced free fatty acid release from rabbit adipose tissue and the accompanying vasodilatation. In two of these experiments nicotinic acid failed to inhibit either the lipolysis or the vascular change. However, in the two experiments in which the infusion of nicotinic acid greatly reduced free fatty acid release, there was a corresponding reduction in the vasodilatation: Fig. 6 illustrates the results of one of these experiments. Nicotinic acid itself caused vasodilatation in the adipose tissue but this effect disappeared soon after the end of the infusion.

Discussion

The anti-inflammatory agents indomethacin and aspirin are known to inhibit the formation of prostaglandin (Vane, 1971). The present finding that these substances have a pronounced inhibitory effect on the vasodilatation accompanying lipolysis, lends strong support to the view that functional vasodilatation in adipose tissue is mediated by a prostaglandin, probably prostaglandin E_2 (Lewis & Matthews, 1969, 1970; Bowery *et al.*, 1970). The experiments showed that indomethacin suppressed prostaglandin formation in adipose tissue to such an extent that when the tissue was activated by $ACTH^{1-24}$ only 36% of the normal amount of prostaglandin E could be extracted from the fat depot. These suppressed values were of the same order as those found in control resting fat tissue in animals which had not been treated with indomethacin (Bowery *et al.*, 1970).

When sufficient time was allowed between infusions of $ACTH^{1-24}$ repeated vasodilator responses could be obtained, but after infusions of indomethacin the response was considerably reduced. This inhibition was due to the reduced formation of prostaglandin since the responses to prostaglandin itself as well as to other vasodilators was not affected unless particularly high doses of indomethacin were used. The concentrations of indomethacin required to inhibit prostaglandin formation and functional vasodilatation in the present experiments are in good agreement with those originally found by Vane (1971) to inhibit the conversion of arachidonic acid to prostaglandin by lung homogenate. Vane found that indomethacin 0·5 to 1 μg/ml in the incubation mixture produced over 50% inhibition of prostaglandin formation. Lewis & Matthews (1968) showed that the blood flow through the rabbit epigastric fat depot is $8{\cdot}07 \pm 1{\cdot}99$ (ml/100 g)/min, which gives the flow through a normal sized fat pad as about 1 to 2 ml/minute. In the present experiments infusion of indomethacin 10–100 (μg/0·1 ml)/min was found to be effective in inhibiting prostaglandin formation and functional vasodilatation. The concentration of the inhibitor reaching the adipose tissue therefore agrees well with that found by Vane (1971) to be effective *in vitro*. It appears, however, that indomethacin produces a cumulative effect in adipose tissue, since the duration of infusion and number of infusions given during a limited time are factors in determining the degree of inhibition of prostaglandin formation and vasodilatation in the gland.

There are two possible origins for the unsaturated fatty acids which are converted to prostaglandins as indicated in Figure 7. They could be derived either

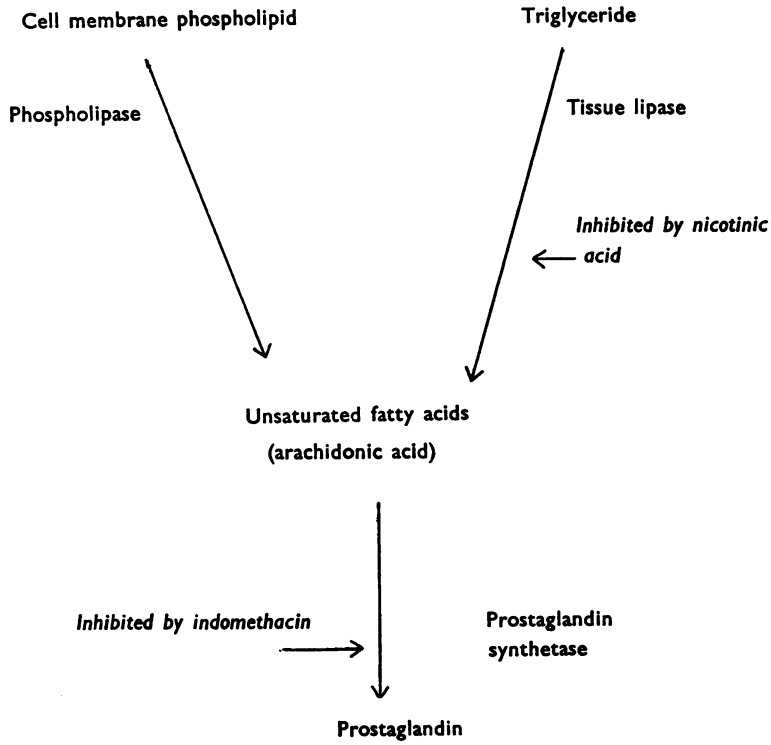

FIG. 7. Prostaglandin formation in adipose tissue.

from the phospholipids of the cell membranes in the adipose tissue or from triglycerides in the fat depot. The present finding that, at least in some experiments, when nicotinic acid inhibits lipolysis, it also inhibits the functional vasodilatation suggests that the unsaturated fatty acids orginate in the triglycerides which are stored in the fat depot. This result does not agree with a recent finding of Mjös & Akre (1971). They found that nicotinic acid prevented noradrenaline-induced free fatty acid release, but did not reduce the accompanying vasodilatation. However, when lipolysis is induced by infusion of catecholamines or sympathetic nerve stimulation the vascular changes are complicated by the direct action of the catecholamines on the blood vessels as shown by Scow (1965); Orö, Wallenberg & Rosell (1965); and Ngai *et al.* (1966). It is possible in the experiments of Mjös & Akre that at least part of the vasodilatation was due to the direct action of catecholamines on β-adrenoceptors in blood vessels, an action which would not be modified by nicotinic acid.

Although indomethacin inhibits the formation of prostaglandin from unsaturated fatty acids such as arachidonic acid, it does not influence the formation of free fatty acids during lipolysis. Therefore there would probably be an accumulation of arachidonic acid or its oxidation products in adipose tissue after treatment with indomethacin, although no chemical measurements of arachidonic or its derivatives have been made.

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(*Received August* 17, 1972)