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EFFECTS OF AN ANTITUMOURAL RHODIUM COMPLEX ON THIOACETAMIDE-INDUCED LIVER TUMOUR IN RATS

CHANGES IN THE ACTIVITIES OF ORNITHINE DECARBOXYLASE, TYROSINE AMINOTRANSFERASE AND OF ENZYMES INVOLVED IN FATTY ACID AND GLYCEROLIPID SYNTHESIS

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Abstract—Rats were injected daily for 8 weeks with 50 mg of thioacetamide per kg to produce liver tumours. Some of these rats were given three doses of 50 mg of an antitumoural Rh(III) complex/kg at 14, 9 and 5 days before the end of the thioacetamide treatment. Thioacetamide decreased the rate of weight gain of the rats and the Rh(III) complex partly restored it. The activities of ATP citrate lyase, acetyl-CoA carboxylase and fatty acid synthetase in the livers were decreased by thioacetamide treatment and the Rh(III) complex partly reversed this effect. By contrast the activity of malic enzyme was increased by both thioacetamide and the Rh(III) complex and this effect probably relates to NADPH production for detoxification rather than for lipogenesis. Treatment with thioacetamide increased the rate of synthesis of di- and triacylglycerols from glycerol phosphate by liver homogenates, the activity of phosphatidate phosphohydrolase and the incorporation of [^{3}H]glycerol into liver triacylglycerol *in vivo*. The Rh(III) complex did not produce a significant reversal of these effects of thioacetamide on glycerolipid synthesis. The total uptake of intraportally injected [^{3}H]glycerol by the livers of thioacetamide treated rats was decreased and this was associated with a lowered activity of glycerol kinase. Thioacetamide increased the activity of hepatic ornithine decarboxylase by about 40-fold, but the Rh(III) complex did not reverse this effect. However, the decrease in tyrosine aminotransferase activity that was produced by thioacetamide was partly reversed by the Rh(III) complex. These results are discussed in relation to the tumour-promoting effects of thioacetamide and the antitumoural action of the Rh(III) complex.

Thioacetamide is a well known hepatotoxic agent that produces liver tumours [1, 2]. Rats were treated with this compound and they were then used to study the action of a Rh(III) complex which had the formula $[Rh(L)_4Cl_2]$ Cl^- in which L = sulphaquinoxaline. This is mild antitumoural agent [3] with an LD_{50} of 300 mg/kg. It is related to a series of platinum and rhodium complexes that exhibit antibacterial and carcinostatic activities [3–7]. The purpose of the present experiments was to see if the Rh(III) complex could be used to alleviate some of the consequences of the neoplastic changes induced by thioacetamide.

This was assessed by determining the effects of the treatments on some aspects of the function of the liver. Furthermore, the activity of several enzymes was determined to study the pattern of change that is produced by thioacetamide and to see whether its effects can be partially reversed by the Rh(III) complex. These measurements concentrated on enzymes involved in the synthesis of fatty acids and glycerolipids in the liver. We were particularly interested to determine the effects of the hepatocarcinogenicity of thioacetamide on the synthesis of membrane lipids. In addition, the changes in activities of tyrosine aminotransferase, ornithine decarboxylase and phosphatidate phosphohydrolase were compared since these activities are known to increase in conditions including metabolic stress and toxicity.

MATERIALS AND METHODS

Animals and their treatment. Male albino Wistar rats were maintained in a standard laboratory diet (Sanders, H-28), with food and water *ad libitum*. They were injected each day with a freshly prepared solution of thioacetamide in 0.15 M NaCl (20 mg/ml i.p.) at a dose of 50 mg/kg for an 8 week period. Control rats received the equivalent volume of 0.15 M NaCl. At about 14 days before the end of the treatment with thioacetamide, six rats of each group (control and thioacetamide-treated) were injected with a sonicated suspension of the Rh(III) complex in 0.15 M NaCl at a dose of 50 mg/kg (i.p.), which was repeated 5 and 10 days later. Therefore each rat received three doses of Rh(III) complex. The use of this regimen and dose correspond to the low solubility of the complex, with the dose administered an interval of 5 days is necessary for the complex to be gradually absorbed. At the end of the treatment four rats of every experimental group were decapitated and the livers were obtained.

The livers were normally homogenized with five strokes up and down in 4 vol. of ice-cold 0.25 M sucrose, 20 mM HEPES, adjusted to pH 7.4 with KOH, and containing 0.2 mM dithioerythritol when enzymes of glycerolipid synthesis were determined. Glycerol phosphate acyltransferase and the incorporation of glycerol phosphate into neutral lipids were measured in whole homogenates. The homogenising medium used for the lipogenic enzymes contained 20 mM Tris–HCl, pH 7.4, instead of HEPES. When ornithine decarboxylase and tyrosine aminotransferase activities were determined, the latter medium was fortified with 5 mM dithioerythritol and 0.2 mM pyridoxal phosphate. The homogenates were then centrifuged at 4° for 10 min at 18,000 *g* and the supernatant was collected. Microsomal and cytosolic fractions were then prepared by centrifuging at 105,000 *g* for 45 min at 4° (r_{max} = 8.14 cm). When required the microsomal fractions were resuspended in 0.25 M sucrose containing 0.2 mM dithioerythritol and 20 mM HEPES buffer ph 7.4.

The incomplete recovery of the endoplasmic reticulum in the microsomal fraction was compensated for by determining the activity of arylesterase in this fraction relative to the total homogenate. The latter enzyme was used as a marker for the endoplasmic reticulum.

In order to estimate the rate of glycerolipid synthesis in the liver *in vivo*, anaesthetized rats were injected in the portal vein with 0.188 mM [1.3–^{3}H]-glycerol (1.8 Ci/mol) and 0.212 mM potassium palmitate in 0.16 M NaCl containing 1.8 mg of fatty acid poor bovine serum albumin per ml. The method and those of the extraction and analysis are based on the methods described by Brindley *et al.* [8].

Analytical methods. Protein concentrations were determined either by the method of Lowry *et al.* [9] or Bradford [10] except that Brilliant Blue G was employed in the latter case. DNA was determined by the method of Burton [11]. The techniques used for measuring enzyme activities were essentially described as follows: malic enzyme [12], ATP-citrate lyase [13], acetyl-CoA carboxylase [14], fatty acid synthetase [15], glycerol phosphate acyltransferase and the incorporation of glycerol phosphate into neutral lipid [16], phosphatidate phosphohydrolase with a final concentration of 5 mM $MgCl_2$ [17], glycerol kinase [18], tyrosine aminotransferase [19], lactate dehydrogenase [20] and arylesterase [21]. The activity of ornithine decarboxylase in the cytosol was determined by monitoring the release of $^{14}CO_2$ from DL-[1-^{14}C] ornithine [22, 23]. Each assay contained in a final volume of 0.1 ml: 100 mM Tris, adjusted to pH 7.4 with HCl, 5 mM dithioerythritol, 0.2 mM pyridoxal phosphate, and 0.2 mM DL-[1-^{14}C] ornithine (10 Ci/mol). The incubations were for 1 hr at 37° and they were stopped by injecting 0.5 ml of 4.65 M $HClO_4$ through a Subaseal which closed the flasks. Hyamine hydroxide (0.4 ml, diluted 1:1 with water) was injected into an Eppendorf tube suspended in the flasks and $^{14}CO_2$ was collected by shaking for 1 hr at 37°. A sample of the hyamine hydroxide was removed and ^{14}C was determined.

Materials. Radiochemicals were purchased from Amersham International and the sources of the reagents have been described previously [3, 16, 17].

RESULTS

Body and liver weights

At the beginning of the experiment the rats weighed 180–220 g. Injection of thioacetamide decreased the body weight gain as observed previously [24]. The administration of the Rh(III) complex towards the end of the treatment period significantly improved the weight gain (Table 1). On its own the Rh(III) complex had no significant effect of weight gain.

The livers of the rats treated with thioacetamide were heavier than those of the controls as expected from previous work [25, 26]. The Rh(III) complex did not significantly alter the liver weight either in the presence or absence of thioacetamide. The amount of protein per g of liver was decreased in rats treated with thioacetamide whereas that of DNA was increased (Table 1).

Table 1. Effects of thioacetamide and the Rh(III) complex on the body weight, liver weight and liver composition of rats

Treatment	Body weight (g)	Liver weight (g)	$\frac{\text{Liver wt} \times 100}{\text{Body wt}}$	Liver protein (mg/g)	Liver DNA (mg/g)
I Control	352 ± 27	12.0 ± 0.87	3.42 ± 0.17	108.66 ± 15.2	9.98 ± 1.50
II Rh(III) complex	358 ± 54	12.5 ± 2.2	3.55 ± 0.22	109.30 ± 11.8	11.00 ± 1.45
III Thioacetamide	246 ± 31 I vs III***	13.4 ± 2.1 I vs III*	5.44 ± 0.60 I vs III***	94.00 ± 14.8 I vs III*	14.70 ± 1.54 I vs III***
IV Thioacetamide + Rh(III) complex	275 ± 30 I vs IV*** II vs IV*** III vs IV*	14.2 ± 1.8 I vs IV*** II vs IV*	5.15 ± 0.58 I vs IV*** II vs IV***	100.90 ± 18.1	13.40 ± 1.93

Rats were treated with thioacetamide or the Rh(III) complex as described in the Material and Methods section. The results are from 15 rats per group except for the DNA values where there are 9 rats per group. The significance of the differences between the groups is shown by: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 2. Effects of thioacetamide and the Rh(III) complex on the hepatic activities of enzymes connected with fatty acid biosynthesis

Treatment	Activities (μmol. min^{-1}.g of liver^{-1})			
	Malic enzyme (3)	ATP citrate lyase (3)	Acetyl:CoA carboxylase (4)	Fatty acid synthetase (3)
I Control	1.06 ± 0.092	0.895 ± 0.083	0.153 ± 0.011	0.344 ± 0.021
II Rh(III) complex	2.02 ± 0.046 I vs II***	1.20 ± 0.14 I vs II*	0.232 ± 0.002 I vs II***	0.361 ± 0.012
III Thioacetamide	2.58 ± 0.023 I vs III***	0.762 ± 0.01	0.078 ± 0.010 I vs III***	0.226 ± 0.005 I vs III**
IV Thioacetamide + Rh(III) complex	3.06 ± 0.60 I vs IV** II vs IV*	0.936 ± 0.046 II vs IV* III vs IV**	0.104 ± 0.015 I vs IV** II vs IV*** III vs IV*	0.234 ± 0.009 I vs IV** II vs IV***

The number of rats indicated in parenthesis was treated as described in the Materials and Methods section and the significance of the differences between the groups is given by: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Enzymes associated with fatty acid biosynthesis

Treatment of the rats with thioacetamide and the Rh(III) complex increased the activity of malic enzyme (Table 2). The activities of acetyl-CoA carboxylase and fatty acid synthetase per g of liver were decreased in rats treated with thioacetamide. This decrease, was not compensated for by the increase in liver weight. The Rh(III) complex increased the activities of ATP: citrate lyase in the presence or absence of thioacetamide. The activity of malic enzyme was also increased in rats treated with the Rh(III) complex and thioacetamide either alone, or in combination.

Ratio of glycerolipid synthesis and the activities of glycerol phosphate acyltransferase and phosphatidate phosphohydrolase

Treatment of the rats with thioacetamide or the Rh(III) complex had no significant effect on the total activity of glycerol phosphate acyltransferase in the liver homogenates (Table 3). However, there was an increased incorporation of glycerol phosphate into neutral lipids (diacylglycerol and triacylglycerol), in both groups of rats treated with thioacetamide. This indicates an increase in the activity of phosphatidate phosphohydrolase, which was confirmed when this activity was determined directly. These increases were seen in the total phosphohydrolase activity and that found in the microsomal fraction.

Estimates were made of the rates of triacylglycerol synthesis in the livers of the rats *in vivo* by following the incorporation of [^{3}H]glycerol (Table 4). These results also demonstrate a relative increase in the synthesis of triacylglycerol in rats treated with thioacetamide, irrespective of whether the Rh(III) complex was administered. There were no significant changes in the relative rates of incorporation of [^{3}H]-glycerol into phosphatidylcholine and phosphatidylethanolamine.

Table 3. Effects of thioacetamide and the Rh(III) complex on the hepatic activities of glycerol phosphate acyltransferase and phosphatidate phospholydrolase

Treatment	Activities (nmol.min^{-1}.g of liver^{-1})				
	Glycerol phosphate acyltransferase (3)	Neutral lipid from glycerol phosphate (3)	Phosphatidate phosphohydrolase		
			Soluble (6)	Microsomal (6)	Total (6)
I Control	105 ± 7.0	10.3 ± 1.5	58.3 ± 12	11.2 ± 4.4	69.5 ± 15
II (Rh(III) complex	104 ± 5.3	9.60 ± 2.5	64.7 ± 18	10.8 ± 5.7	75.5 ± 23
III Thioacetamide	90.2 ± 7.5	20.5 ± 3.0 I vs III**	84.4 ± 24 I vs III*	23.0 ± 6.7 I vs III**	107.0 ± 31 I vs III*
IV Thioacetamide + Rh(III) complex	91.1 ± 12.5	22.7 ± 4.3 I vs IV** II vs IV*	105.0 ± 22 I vs IV** II vs IV**	20.9 ± 4.7 I vs IV** II vs IV**	126.0 ± 25 I vs IV*** II vs IV**

The number of rats indicated in parenthesis was treated as described in the Material and Methods section and the significance of the differences between groups is given by: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. The activity of glycerol phosphate acyltransferase was determined by measuring the total incorporation of [^{3}H]glycerol 3-phosphate into lipids in the presence of a palmitoyl-CoA generating system [16].

Table 4. Effect of thioacetamide and Rh(III) complex on the hepatic synthesis of glycerolipids from [^{3}H]glycerol injected *in vivo*

	d.p.m. × 10^6		Relative incorporation (%)				
	Total radioactivity (^{3}H/g of liver)	Radioactivity incorporation into lipid (^{3}H/g of liver)	Phosphatidate	Diacylglycerol	Triacylglycerol	Phosphatidylcholine	Phosphatidylethanolamine
I Control (3)	8.07 ± 2.39	0.352 ± 0.124	13.6 ± 5.9	41.9 ± 3.8	13.1 ± 4.2	17.9 ± 5.1	11.4 ± 3.9
II Rh(III) complex (3)	7.46 ± 4.25	0.339 ± 0.162	21.1 ± 5.6	40.8 ± 4.4	7.5 ± 5.0	18.5 ± 3.0	9.1 ± 1.9
III Thioacetamide (4)	4.15 ± 1.30 I vs III*	0.238 ± 0.070	16.7 ± 5.0	41.7 ± 3.0	23.7 ± 15.6	16.6 ± 3.0	8.1 ± 2.2
IV Thioacetamide + Rh(III) complex (4)	3.35 ± 3.18	0.287 ± 0.234	13.7 ± 2.1	31.9 ± 2.6 I vs IV** II vs IV* III vs IV**	24.1 ± 2.8 I vs IV** II vs IV**	18.4 ± 2.1	7.7 ± 1.4

The number of rats indicated in parenthesis was treated as described in the Materials and Methods section and the significance of the difference between groups is given by: *$P < 0.05$, **$P < 0.01$.

Table 5. Effects of thioacetamide and the Rh(III) complex on the hepatic activities of glycerol kinase, ornithine decarboxylase, tyrosine aminotransferase, lactate dehydrogenase and arylesterase

Treatment	Glycerol kinase (3)	Ornithine decarboxylase (3)	Tyrosine aminotransferase (3)	Lactate dehydrogenase (12)	Arylesterase (9)
I Control	8.02 ± 0.37	54.3 ± 18	4.50 ± 0.83	499 ± 121	74.9 ± 4.08
II Rh(III) complex	7.52 ± 0.44	50.0 ± 9.9	4.26 ± 0.51	474 ± 108	64.4 ± 9.82 I vs II**
III Thioacetamide	5.57 ± 0.17 I vs III***	2123 ± 249 I vs III***	2.88 ± 0.56 I vs III*	430 ± 100	22.3 ± 3.27 I vs III***
IV Thioacetamide + Rh(III) complex	5.78 ± 0.53 I vs IV** II vs IV*	2251 ± 407 I vs IV*** II vs IV***	5.44 ± 0.84 III vs IV*	495 ± 136	24.7 ± 4.37 I vs IV*** II vs IV***

The number of rats indicated in parenthesis was treated as described in the Material and Methods section and the significant of the differences between the groups is given by: *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$. The activities of glycerol kinase, lactate dehydrogenase and arylesterase are expressed as μmol of substrate converted. min^{-1}.g of liver $^{-1}$; ornithine decarboxylase in pmol. min^{-1}.g of liver^{-1} and tyrosine aminotransferase in nmol.min^{-1}.g of liver^{-1}.

Glycerol kinase, ornithine decarboxylase, tyrosine aminotransferase, lactate dehydrogenase and arylesterase

Treatment of the rats with thioacetamide decreased the activity of glycerol kinase in the liver (Table 5), and this was paralleled by a decrease in the uptake of [^{3}H]glycerol by the liver when this was injected intraportally (Table 4).

The most dramatic change in enzyme activities was the increase of about 40-fold in the activity of ornithine decarboxylase after treating the rats with thioacetamide (Table 5). This effect was not reversed by the Rh(III) complex. Tyrosine aminotransferase activity was decreased in rats treated with thioacetamide alone, but this effect was reversed by the Rh(III) complex.

Lactate dehydrogenase and arylesterase were used as markers for the cytosolic and endoplasmic reticulum fractions respectively. Neither thioacetamide, nor the Rh(III) complex altered the activities of lactate dehydrogenase. Thioacetamide did decrease the activity of arylesterase (Table 5).

DISCUSSION

Treatment of the rats with thioacetamide decreased the activities of ATP citrate lyase, acetyl-CoA carboxylase and fatty acid synthetase (Table 2). This result is compatible with the decreased incorporation of ^3H_2O into fatty acids that is observed in rats after long-term administration of thioacetamide [24]. By contrast, the activity of malic enzyme increased after thioacetamide treatment (Table 2). This may be related to the increased activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and the generally increased capacity for NADPH production which is probably used in detoxification reactions rather than for fatty acid synthesis [25].

The uptake by the liver of [^{3}H]glycerol that was injected intraportally appeared to be decreased by thioacetamide (Table 4), and this could have resulted in part from the decreased activity of glycerol kinase (Table 5) or a defect in the vascular supply to the liver caused by the tumour. The total (mitochondrial plus microsomal) glycerol phosphate acyltransferase, which is responsible for diverting glycerol phosphate into glycerolipid synthesis, was not significantly altered by thioacetamide treatment (Table 3). However, the activity of phosphatidate phosphohydrolase which helps to control the rate of neutral lipid synthesis was increased by thioacetamide (Table 3) as was the proportion of [^{3}H]glycerol incorporated in triacylglycerol *in vivo* (Table 4).

It is not certain whether this increased capacity of phosphatidate phosphohydrolase was expressed and responsible for the relative increased synthesis of triacylglycerol, since there was no marked change in the relative accumulation of [^{3}H]glycerol in phosphatidate (Table 4). The relative increase in triacylglycerol synthesis could have resulted from an increased availability of fatty acids and an increased activity of diacylglycerol acyltransferase. The short-term administration of thioacetamide is known to increase the accumulation of triacylglycerols in the liver, and this could be a combination of an increased synthesis caused by fatty acid mobilization, and a decrease in the secretion of very low density lipoproteins [27].

The total activity of phosphatidate phosphohydrolase in the liver is thought to be controlled by the balance between the actions of insulin, glucagon and glucocorticoids [28]. Glucagon (through cyclic AMP) and glucocorticoids increase the activity of the phosphohydrolase, probably by stimulating its synthesis, and insulin antagonizes these actions [29, 30].

The effects of metabolic stress (i.e. an increase in the effects of stress hormones relative to insulin) probably account for the increased activity of phosphatidate phosphohydrolase that can accompany a variety of toxic fatty livers [28]. The expression of the increased phosphohydrolase activity is thought to depend upon its translocation from an inactive reservoir in the cytosol, to the membranes of the endoplasmic reticulum where its substrate, phosphatidate is generated [28]. This is promoted by fatty acids [31, 32] which would be released in stress by lipolysis in adipose tissue. It is significant to note that the increase in phosphohydrolase activity found in the liver of the thioacetamide-treated rats is accompanied by an increase in the membrane bound activity (Table 3).

The control of the activities of tyrosine aminotransferase [28] and ornithine decarboxylase [33] are probably coordinated in some respects with that of phosphatidate phosphohydrolase, since glucocorticoids and increases in the concentration of intracellular cyclic AMP stimulate their synthesis. Both the activities of ornithine decarboxylase [34] and phosphatidate phosphohydrolase [35] are increased in the liver remnant after partial hepatectomy.

The acute [36, 37] and chronic (Table 5) administration of thioacetamide produced dramatic increases in the order of 50- and 40-fold respectively in the activities of ornithine decarboxylase. These are much higher than those for the phosphohydrolase. The tyrosine aminotransferase activity, in fact, fell when thioacetamide was administered alone (Table 5). Ornithine decarboxylase is known to have very rapid rates of synthesis and degradation compared with other enzymes and this enables fairly rapid changes in the intracellular concentrations of polyamines to occur. Its synthesis [38], like that of tyrosine aminotransferase [39] and phosphatidate phosphohydrolase [40] is inhibited by spermine. In the case of ornithine decarboxylase, this appears to be a feed-back mechanism of control.

Increases in ornithine decarboxylase activity are often associated with the stimulation of cell growth and tumour formation [41, 42] which also requires an increased synthesis of membrane lipids. Polyamines *in vitro* can stimulate the activities of glycerol phosphate acyltransferase [43–45]. It also increases the microsomal phosphatidate phosphohydrolase [46, 47], probably by facilitating the effects of long-chain fatty acid in promoting the translocation of the cytosolic enzyme to the endoplasmic reticulum [48, 49].

It is not yet certain whether these effects of polyamines occur *in vivo*, but the present work does

demonstrate that an increased activity of ornithine decarboxylase is also accompanied by a higher activity of membrane-associated phosphatidate phosphohydrolase (Tables 3 and 5). There was, however, no evidence of an increased synthesis of phospholipids in the liver of thioacetamide-treated rats although triacylglycerol synthesis was raised (Table 4). In other work [50], the long-term administration of thioacetamide decreased the incorporation of ^{32}P in the phosphatidylcholine fraction of liver *in vivo*.

A further objective in this work was to investigate whether the Rh(III) complex could ameliorate some of the biochemical changes that were produced by thioacetamide. Indications that the Rh(III) complex could reverse some of the biochemical changes were obtained for body weight (Table 1), tyrosine aminotransferase (Table 5), ATP citrate lyase, acetyl-CoA carboxylase, and fatty acid synthetase (Table 2). By contrast to the latter three lipogenic enzymes, there was a further increase in the activity of malic enzyme when the thioacetamide-treated rats were also given the Rh(III) complex. This probably related to a further demand for NADPH for detoxification reactions and microsomal electron transport. In further unpublished work we have also demonstrated that the Rh(III) complex can partly reverse the thioacetamide-induced changes in the content of alkaline phosphatase, aspartate aminotransferase, glutamate dehydrogenase, γ-glutamyltransferase, uric acid and bilirubin in the blood and that of uric acid, bilirubin and creatinine in the urine of rats.

The mechanisms by which the antitumoural Rh(III) complex might counteract the metabolic effects of thioacetamide are not yet established. Thioacetamide is thought to induce the formation of humoral growth factors and to stimulate DNA synthesis and mitosis in the rat [51]. Conversely, the antitumoural complexes of Rh and Pt have an antimitotic effect; they inhibit cell growth but they do not appear to alter essential metabolic activity [4]. A primary lesion of nuclear DNA function that seems to appear in the case of Rh(III) complexes [52] could halt further DNA synthesis and cell proliferation. It is hoped that the present work may provide further insight into the mechanisms of tumour formation that is provoked by thioacetamide, and the antitumoural effect of Rh(III) complexes.

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REFERENCES

1. G. O. Fitzhugh and A. A. Nelson, *Science (Washington)* **108**, 626 (1948).
2. M. Cascales, E. M. Robles-Chillida, B. Feijóo, S. Cerdán, P. Martín-Sanz and A. Santos-Ruiz, *Rev. Esp. Fisiol.* **38**, suppl., 105 (1982).
3. D. G. Craciunescu, C. Ghirvu and A. Doadrio, *An. Real Acad. Farm.* **49**, 515 (1983).
4. J. Dehand, J. Jordanov and J. P. Beck, *Chem.-Biol. Interact.* **11**, 605 (1975).
5. A. Erck, E. Sherwood, J. L. Bear and A. P. Kimball, *Cancer Res.* **36**, 2204 (1976).
6. T. R. Jack, *J. inorg. Biochem.* **12**, 187 (1980).
7. M. J. Cleare, P. C. Hydes and C. F. J. Barnard, *Proc. Johnson Matthey Research Centre* **214** (1983).
8. D. N. Brindley, M. Bowley, S. Burditt, P. H. Pritchard, K. A. Lloyd-Davies and P. Boucrot, *J. Pharm. Pharmc.* **28**, 676 (1976).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 275 (1951).
10. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
11. K. Burton, *Biochem. J.* **62**, 315 (1956).
12. N. Z. Baquer, M. Cascales, P. McLean and A. L. Greenbaun, *Eur. J. Biochem.* **68**, 403 (1976).
13. Y. Takeda, F. Suzuki, K. Adacki and A. Tanioka, *Nutr. Proc. Int. Congress* **8**, 66 (1970).
14. E. M. McNeillie, R. A. Clegg and V. A. Zammit, *Biochem. J.* **200**, 639 (1981).
15. S. Kumar, *Archs Biochem. Biophys.* **183**, 625 (1977).
16. N. Lawson, A. D. Pollard, R. J. Jennings and D. N. Brindley, *FEBS Lett.* **146**, 204 (1982).
17. S. C. Butterwith, R. Hopewell and D. N. Brindley, *Biochem. J.* **220**, 825 (1984).
18. R. Lagunas, P. McLean and A. L. Greenbaum, *Eur. J. Biochem.* **515**, 179 (1970).
19. F. A. O. Marston and C. I. Pogson, *FEBS Lett.* **83**, 277 (1977).
20. E. D. Saggerson and A. L. Greenbaum, *Biochem. J.* **115**, 405 (1969).
21. E. H. Shephard and G. Hübscher, *Biochem. J.* **113**, 429 (1969).
22. M. E. Brosnan, B. V. Roebothan and D. E. Hall, *Biochem. J.* **190**, 395 (1980).
23. L. S. Jefferson and A. E. Pegg, *Biochim. biophys. Acta* **484**, 177 (1977).
24. J. Osada, E. Palacios and M. Cascales, *Rev. Esp. Fisiol.* **38**, suppl., 129 (1982).
25. S. Cerdán, M. Cascales and A. Santos-Ruiz, *Molec. Pharmac.* **19**, 451 (1981).
26. P. Martín-Sanz, R. Santos-Ruiz and C. Cascales, *An. Real Acad. Farm.* **47**, 201 (1981).
27. H. Franke, T. Zimmermann and R. Dargel, *Virchows Archiv. B* **44**, 99 (1983).
28. D. N. Brindley, *Prog. Lipid Res.* **23**, 115 (1984).
29. R. A. Pittner, R. Fears and D. N. Brindley, *Biochem. J.* **225**, 455 (1985).
30. R. A. Pittner, R. Fears and D. N. Brindley, *Biochem. J.* **230**, 525 (1985).
31. C. Cascales, E. H. Mangianpane and D. N. Brindley, *Biochem. J.* **219**, 911 (1984).
32. P. Martín-Sanz, R. Hopewell and D. N. Brindley, *FEBS Lett.* **175**, 284 (1984).
33. Z. N. Canellakis and R. C. Theoharides, *J. biol. Chem.* **251**, 4436 (1976).
34. E. Hölttä, *Biochim. biophys. Acta* **399**, 420 (1975).
35. E. H. Mangiapane, K. A. Lloyd-Davies and D. N. Brindley, *Biochem. J.* **134**, 103 (1973).
36. A. E. Pegg, C. Conover and A. Wrona, *Biochem. J.* **170**, 651 (1978).
37. J. E. Seely and A. E. Pegg, *Biochem. J.* **216**, 701 (1983).
38. J. E. Kay and W. L. Lindsey, *Biochem. J.* **132**, 791 (1972).
39. A. Auberger, M. Samson and A. LeCam, *Biochem. J.* **214**, 679 (1983).
40. R. A. Pittner, R. Fears and D. N. Brindley, *FEBS Lett.* to be submitted.
41. C. W. Tabor and H. Tabor, *An. Rev. Biochem.* **45**, 285 (1976).
42. H. G. Williams-Ashman and Z. N. Canellakis, *Perspectives Biol. Med.* **22**, 421 (1979).

43. S. C. Jamdar, *Archs Biochem. Biophys.* **182**, 723 (1977).
44. S. C. Jamdar, *Archs Biochem. Biophys.* **195**, 81 (1979).
45. E. J. Bates and E. D. Saggerson, *Biochem. Soc. Trans.* **9**, 57 (1981).
46. F. Moller and M. R. Hough, *Biochim. biophys. Acta* **711**, 521 (1982).
47. S. C. Jamdar and L. J. Osborne, *Biochim. biophys. Acta* **752**, 79 (1983).
48. P. Martín-Sanz, R. Hopewell and D. N. Brindley, *FEBS Lett.* **179**, 262 (1985).
49. R. Hopewell, P. Martín-Sanz, A. Martin, J. Saxton and D. N. Brindley, *Biochem. J.* **232**, 485 (1985).
50. E. Palacios, J. Osada, C. Arce, H. Aylagas and A. Santos-Ruiz, *Rev. Esp. Fisiol.* **38**, suppl., 135 (1982).
51. C. G. Morley and J. L. Boyer, *Biochim. biophys. Acta* **477**, 165 (1977).
52. M. J. Cleare, *Coord. Pham. Rev.* **12**, 349 (1974).