in rats undergoing a 6-day treatment with the same dose of morphine [8], in contrast to the acute effect of the analgesic [2].

Increased enzyme synthesis represents a phenomenon of 'enzymatic adaptation', that is, it indicates the capacity of the animals to adapt to changing conditions. Even if, at present, we know little about the purpose of this enzymatic adaptation (also exhibited by tryptophan oxidase), we know that there are at least two independent stimuli for the adaptive response: the corticosteroid and the substrate inductions [6]. A great variety of stimuli, chemical and environmental (the latter generally associated with fear, hunger, pain and cold exposure [4]), give the signal, via the hypothalamic-pituitary system, for corticosteroid secretion thus inducing the enzyme synthesis.

The mechanism by which morphine influences corticosteroid secretion (and consequently TAT synthesis) is not clearly defined, but evidence suggests that it exerts its effect at a central site in the region of the hypothalamus [9], probably through the stimulation and inhibition of the synthesis or output of releasing factors (RFs) responsible for the control of the pituitary-adrenal system[10]. The intervention of morphine on RFs might be direct or indirect through the biogenic amines, since these transmitters are implicated in the mechanism of RFs secretion [11, 12] and evidence exists that their turnover rate is modified by acute and chronic morphine administration [13].

Morphine may also influence TAT synthesis by acting at other sites. It has been shown, for instance, with experiments *in vitro*, that morphine interferes with the corticosteroid-binding capacity of rat liver slices [14].

In any case the present results give evidence of a supplementary pharmacological tool suitable for demonstrating both the adrenal response to narcotics and the onset of a tolerance to their chronic administration.

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Modification of protein–lipid interactions in the Gunn rat by treatment of microsomal UDP-glucuronyltransferase with diethylnitrosamine*

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The activities of UDP-glucuronyltransferases (EC 2.4.1.17) in liver microsomes from homozygous Gunn rats, in contrast to normal rats, are insensitive to treatments which modify the fluidity of the lipid phase of the membrane [1, 2]. The defect in the function of UDP-glucuronyltransferases in homozygous Gunn rats thus includes abnormal regulatory properties as well as abnormal rates of catalysis. Of great potential significance for understanding of the defect in these rats is the fact that the carcinogen diethylnitrosamine (DEN) activates microsomal UDP-glucuronyltransferase (EC 2.4.1.17) [3]. Addition of DEN to microsomes from Gunn rats corrects the deficiencies in the rates of glucuronidation of *o*-aminophenol [3], *o*-aminobenzoate [3] and *p*-nitrophenol.

Since it seems as if DEN corrects the defect in the catalytic function of UDP-glucuronyltransferase from Gunn rats, DEN also might correct the abnormalities of regulatory function, that is, produce a form of the enzyme that is activated on subsequent modification of the fluidity of the membrane lipids by treatment with phospholipases and detergents. This possibility has been investigated by determining whether DEN modifies the effect of lipid–protein interactions on the properties of UDP-glucuronyltransferase in liver microsomes from homozygous Gunn rats.

Homozygous female Gunn rats were obtained from the colony maintained by Dr. M. M. Thaler, University of California Medical Center, San Francisco. Techniques for the preparation of microsomes, and partially purified phospholipase A, and assay methods have been described previously [1]. Details of conditions of treatment of microsomes with detergents and phospholipase A, and conditions for enzyme assays are given in the legends and text. Proteins were measured with the biuret method [4].

Prior treatment of microsomes from Gunn rats with DEN produces a form of UDP-glucuronyltransferase which is activated on subsequent treatment with phospholipase A (Table 1). DEN activates UDP-glucuronyltransferase in microsomes from normal as well as Gunn rats, but does not modify the activating effect of phospholipase A on enzyme from normal rats. Addition of DEN to microsomes from Gunn rats also allowed for activation of UDP-glucuronyltransferase by Triton X-100 (Table 2). The effect of Triton on activity was biphasic (Fig. 1), which is typical of that obtained with normal microsomes. This is a signifi-

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Table 1. Effect of treatment with phospholipase A and DEN on the activity of UDP-glucuronyltransferase*

Additions to Assay	Phospholipase A treatment	Activity: Homozygous Gunn rat	Activity: Normal Wistar rat
None	–	0·52	0·55
None	+	0·52	2·5
DEN	–	0·93	1·4
DEN	+	2·2	3·4

* Initial rates of activity of UDP-glucuronyltransferase were measured as described by Zakim *et al.* [1] at 37°. The concentrations of UDP-glucuronic acid and *p*-nitrophenol were 1·0 and 0·2 mM respectively. Microsomes were treated in 50 mM Tris, pH 8·0, with partially purified phospholipase A at a protein to phospholipase A ratio of 100/1. Treatment was for 30 sec at 25° in the presence or absence of 10 mM DEN. Digestion with phospholipase A was stopped by addition of EDTA. Activities are expressed as nmoles *p*-nitrophenol glucuronidated/min/mg of protein.

Table 2. Effect of treatment with Triton X-100 and DEN on the activity of UDP-glucuronyltransferase*

Additions to assay	Triton treatment	Activity: Homozygous Gunn rat	Activity: Normal Wistar rat
None	–	0·52	0·55
None	+	0·48	2·7
DEN	–	0·93	1·4
DEN	+	3·3	3·7

* Initial rates of activity of UDP-glucuronlytransferase were measured in 50 mM Tris, pH 7·6, 0·2 mM *p*-nitrophenol and 1·0 mM UDP-glucuronic acid. When added, the ratio of Triton to microsomal protein was 1/5 and the concentration of DEN 10 mM.

cant observation, since even high concentrations of Triton X-100 are without effect on the activity of UDP-glucuronyltransferase from Gunn rats in the absence of DEN [2]. In accord with work published previously [5], activation of UDP-glucuronyltransferase by DEN was reversible. Removal of DEN also prevented activation of the enzyme on subsequent treatment with either phospholiphase A or Triton X-100.

The mechanism by which DEN modifies the properties of UDP-glucuronyltransferase in liver microsomes from the Gunn rat is uncertain. It is unlikely that DEN acts simply by correcting the defect in the enzyme, since DEN also modifies the activity of UDP-glucuronyltransferase from normal animals. DEN is also not a non-specific activator of microsomal enzymes, since addition of DEN to microsomes had no effect on the activity of glucose 6-phosphatase, an enzyme which, like UDP-glucuronyltransferase, is sensitive to perturbation of its lipid environment [6]. On the basis of the activities of the DEN-treated enzyme and the effects of treatment with phospholipase A and detergent, it is reasonable to conclude, however, that the conformation of the DEN-treated form of UDP-glucuronyltransferase is similar in normal and Gunn strains of rats, and that the ability of UDP-glucuronyltransferase to detect and react to perturbations of the lipid phase of the membrane depends on a specific conformation of this enzyme, and/or specific types of lipid–protein interactions.

An aspect of this work which is of general interest is that DEN is a carcinogen. In this regard, it is known that the fluidity of the lipid phase of plasma membranes from transformed cells or naturally occurring tumors is altered as compared with that of normal, parent cells [7]. Demonstration that a carcinogen alters lipid–protein interactions within a membrane, hence, may be an important observation relating to the chemical induction of some tumors.

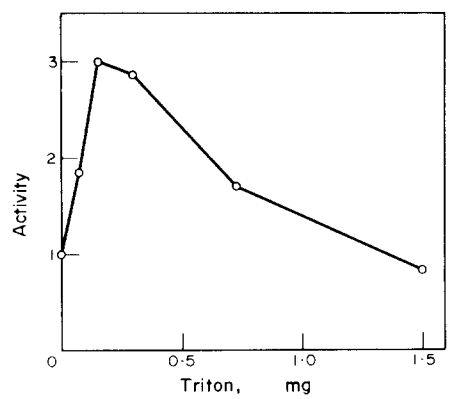

Fig. 1. Effect of Triton X-100 on the activity of UDP-glucuronyltransferase in microsomes from Gunn rats. Initial rates of activity of UDP-glucuronyltransferase were measured as dessribed in Ref. 1 in 50 mM Tris, pH 7·6, 0·2 mM *p*-nitrophenol, 4·0 mM UDP-glucuronic acid and 10 mM DEN; 1·3 mg microsomal protein was added to each assay. The amounts of Triton X-100 present in each assay are indicated above. Activities are expressed as nmoles *p*-nitrophenol glucuronidated/min/mg of microsomal protein.

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Stereospecificity of the microsomal ethanol-oxidizing system*

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The microsomal ethanol-oxidizing system (MEOS) has been interpreted by Lieber and DeCarli [1] to be a unique system contributing significantly to ethanol metabolism *in vivo*. A number of investigators have considered the possibility that MEOS activity is due to catalase or catalase combined with alcohol dehydrogenase activity [2–4], but a number of recent studies support an MEOS function independent of catalase [5–8]. Teschke *et al.* [5, 9] and Mezey *et al.* [10] reported the isolation of a microsomal preparation free of or containing only small amounts of catalase and capable of converting ethanol to acetaldehyde. Thurman and Scholz [3] isolated in a similar manner a catalase-free microsomal fraction, but found it devoid of MEOS activity. Barakat *et al.* [11] examined ethanol oxidation by components of rat liver microsomes and found that highly purified cytochrome P-450 did not oxidize ethanol appreciably.

Alcohol dehydrogenase shows stereospecificity, oxidizing the *R* hydrogen of ethanol in the formation of acetaldehyde [12]. Corrall *et al.* [13] reported that catalase shows the same stereospecificity. This is a report of a study to determine if, in the oxidation of ethanol by MEOS, the *R* or *S* hydrogen is retained in the acetaldehyde formed.

(*R*)-[1-$^3H_1$]ethanol and (*S*)-[1-$^3H_1$]ethanol were prepared and [2-^{14}C]ethanol was purchased from New England Nuclear Corp., Boston, Mass. Initially, media as described by Isselbacher and Carter [14] and Lieber and DeCarli [15] were employed. Isselbacher and Carter incubated ethanol with microsomal material in the presence of NADPH, magnesium chloride, and a sodium phosphate buffer at pH 7·4, while Lieber and DeCarli employed a potassium phosphate buffer at pH 7·4 containing magnesium chloride, an NADPH generating system consisting of NADP, sodium isocitrate and isocitrate dehydrogenase, and in the presence of nicotinamide. All later incubations were with a potassium phosphate buffer (80 mM) at pH 7·4 with 0·3 mM NADPH and 5 mM magnesium chloride.

Microsomes incubated in the medium of Isselbacher and Carter [14] were prepared from a 20% liver homogenate in 0·25 M sucrose made from livers of female Sprague–Dawley rats killed by decapitation. The microsomal fraction collected after centrifugation at 105,000 *g* was resuspended in 0·15 M KCl [14, 16]. Using the medium of Lieber and DeCarli [15] microsomes were prepared by homogenization in 0·15 M KCl and, after centrifugation at 9000 *g* for 30 min, the crude microsomes were collected by centrifugation at 105,000 *g* and resuspended in KCl and incubated, or further purification was attempted by washing. For the latter, the KCl suspension was centrifuged again at 105,000 *g*. The resulting pellet was resuspended in KCl, followed by a third centrifugation at 105,000 *g* and then resuspension of the resulting pellet in KCl for incubation. Protein in the final microsomal suspension was determined using biuret [17].

Livers were obtained from three monkeys. The first, a male, had been fasted 72 hr and his liver was removed under phencyclidine anesthesia. The other two were females which had been fasted overnight and were under barbiturate anesthesia. The first monkey, 1 yr before sacrifice, was under hyperthermia for 48 hr for another study. The second monkey was being sacrificed after accidental hemorrhage in preparation for another study; the third monkey had been oophorectomized and the liver was removed at the time its brain was being isolated and placed under perfusion for another study. It is assumed that these circumstances do not alter the stereospecificity of ethanol oxidation, but could alter the quantity of ethanol utilized. The use of these monkeys was preferred to killing of monkeys for the present study. The livers were collected in cold isotonic saline and homogenized within 40 min of removal.

To each of two flasks were added the potassium phosphate buffer, magnesium chloride and NADPH to give the final concentrations noted, and the microsomal suspension to give a final protein concentration of 3 mg/ml. The flasks were stoppered with inlets in the stoppers to permit subsequent injections into the media. The gas phase was air, except that oxygen was used when the conditions of Isselbacher and Carter [14, 18] were employed. Flasks with contents were incubated for 10 min at 37°, and then labeled ethanol was injected. To each flask, 0·4 to 1·2 μCi of [2-^{14}C]ethanol was added and 1–3 μCi of (*R*)-[1-$^3H_1$]ethanol was added to one flask and 1–3 μCi of (*S*)-[1-$^3H_1$]ethanol to the other flask. The ethanol was added to give a final concentration in the 40-ml volume of 2·3 mg/ml. Incubation was for 20 min. Two additional flasks serving as controls were incubated identically, except that that the microsomal suspension was boiled before addition or KCl was substituted for the suspension.

Incubation was terminated by the addition of equal volumes 0·3 N $Ba(OH)_2$ and 5% $ZnSO_4$ to the flasks and then 4·8 mg of unlabeled acetaldehyde in water was injected. The content of each flask was cooled and centrifuged. The supernatant was distilled and the initial distillate was collected in dimetol reagent. The dimetoacetaldehyde that precipitated was collected, washed, dried and weighed [12].

Catalase-free MEOS was prepared essentially as described by Mezey *et al.* [10], except that female Sprague Dawley rats [9] weighing 200–300 g were used. After isolation of the microsomal pellet and its solubilization, an ammonium sulphate precipitate was dialyzed. The dialyzed protein was passed through a Sephadex-G-25 column and was then placed on a DEAE cellulose column and eluted with KCl [10]. Catalase activity appeared at the beginning

* A preliminary account of these studies has appeared (*Biochem. Soc. Transactions* **2**, 994 1974).