amiton, which evoke the static tremor and increase the amount of acetylcholine in central nervous system, also decreased the concentration of total flavines in rats brain as oxotremorine did.[6] These decreases could be prevented by the application of atropine. On the other hand, physostigmine produced the decrease of glycogen content in cortical and subcortical brain structures in the rat.[7] This glycogenolytic effect of physostigmine could be blocked with atropine, as well as with propranolol. Beta-adrenergic blocking drug propranolol also prevented the static tremor produced with oxotremorein.[8]

The aim of this work was to find whether oxotremorine has any influence on glycogen concentration in various brain structures of the rat.

The experiments were carried out on adult Mill-Hill rats. Oxotremorine (kindly donated by Professor P. Stern, Institute of Pharmacology, Medical Faculty, Sarajevo, Yugoslavia) was administrated intravenously in a dose of 0·25 mg/kg. Atropine sulphate (0·5 mg/kg) was administrated intraperitoneally 20 min and propranolol (10 mg/kg) 30 min before oxotremorine.

From the frozen brain tissue, glycogen was extracted by the method of Le Baron[9] and after purification from the water solution was determined by spectrophotometric method of Montgomery.[10]

The results obtained are presented in Table 1.

Our data show that atropine, as well as propranolol prevents the glycogenolytic effect of oxotremorine in rat brain. The results are similar with those obtained with physostigmine.[7] On the other hand, Hutchins and Rogers[11] did not find any effect of physostigmine and oxotremorine on glycogen concentration in whole mouse brain. Such results are not unexpected because, there are differences in metabolism of oxotremorine in rat and mouse which produce the difference in pharmacological activities.[12]

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Lipid peroxide formation in experimental inflammation*

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ANTI-INFLAMMATORY drugs have been reported to inhibit cellular oxidation and thus interfere with energetics.[1] Concurrent effects, if any, of these drugs on lipid peroxidation arising out of the resultant disturbances in the redox values and the consequent autocatalytic disruption of membrane systems

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have, however, not received any attention. Lipid peroxidation is known to occur in a variety of pathological conditions including irradiation sickness.[2] Although u.v. induced erythemia is employed as a routine method for eliciting inflammatory response in experimental animals, the correlation of lipid peroxidation to the aetiology of inflammation has remained unexplored.

Results are presented in this communication to demonstrate that inflammation induced by carrageenin in albino mice is accompanied by a significant increase in the *in vitro* output of lipid peroxides by liver. Curcumin (the anti-inflammatory principle of *Curcuma indica*) and phenylbutazone inhibit both oedema development and lipid peroxide accumulation.

Albino mice (20–25 g weight) drawn from the stock Colony of this Institute were starved overnight. To their left paws were administered with the aid of a sterile Micro-Alga syringe 0·025 ml of 1% w/v solution of carrageenin in 150 mM NaCl. Control mice received in their left paws an equivalent volume of 150 mM NaCl. The difference in weight between left and right paws gave a measure of the inflammation produced. One hr prior to injecting carrageenin, the drugs under test were administered and at the required time intervals they were sacrificed, tissues quickly excised out and washed with chilled 150 mM NaCl. Ten % w/v suspensions of tissues were made by grinding them with 150 mM KCl in a Potter-Elvehjem tissue homogenizer with a Teflon pestle. Homogenates were incubated at 37 ± 1° in a metabolic shaker at 120 horizontal strokes (amplitude 10 cm) per min and aliquots assayed at hourly intervals for the formation of lipid peroxides by the thiobarbituric acid method as described earlier.[3] Thiobarbituric acid values were converted to μmoles of malonyldialdehyde by using a molar extinction coefficient value 156×10^5 as given by Utley *et al.*[4] Curcumin was supplied by Dr. N. M. Khanna of this Institute and phenylbutazone was a gift from Suhrid Geigyl Ltd., Baroda, India. Rutin was a chemically pure commercial preparation. Typical results of lipid peroxide accumulation and oedema development in mice are given in Table 1. Almost similar results (not included in this paper) were obtained in albino rats in which oedema was induced by formaldehyde. Brain and kidney homogenates did not show any increased output of lipid peroxides as a result of oedema whereas liver showed a high increase in lipid peroxide 3 hr after carrageenin administration (Fig. 1). Maximum oedema production was noticed within 3–6 hr. The output of lipid peroxides in liver, however, touched a high value at 3 hr and then rapidly declined to levels comparable to the controls.

TABLE 1. OEDEMA AND LIPID PEROXIDE FORMATION BY TISSUE HOMOGENATES INDUCED BY CARRAGEENIN IN ALBINO MICE

Time elapsed after carrageenin administration (hr)	Oedema* (mg)	Lipid peroxide formation†		
		Liver	Kidney	Brain
0	nil	12·8 ± 0·25	5·3 ± 0·10	12·1 ± 0·20
1	nil	12·8 ± 0·30	5·4 ± 0·15	11·9 ± 0·20
2	36	15·4 ± 0·25	4·2 ± 0·15	12·1 ± 0·20
3	84	19·7 ± 0·30	4·4 ± 0·15	11·5 ± 0·15
4	88	13·8 ± 0·30	5·3 ± 0·15	11·3 ± 0·15
6	85	12·8 ± 0·25	5·0 ± 0·15	12·2 ± 0·15
24	22	12·8 ± 0·20	5·0 ± 0·20	12·3 ± 0·15
Control animals	nil	12·4 ± 0·20	5·5 ± 0·20	12·1 ± 0·15

* Oedema is expressed as weight difference in mg between the left and right paw of the animal.

† Lipid peroxide is expressed as μmoles malonyldialdehyde produced in 3 hr by 1 mg of fresh weight of tissue.

Data given are the means with standard error of six animals in each set.

The effects of pretreatment of mice with the drugs are summarized in Table 2. Curcumin and phenylbutazone were very effective in arresting the development of oedema. Liver homogenates of mice which had received phenylbutazone and no carrageenin produced less peroxides than mice which

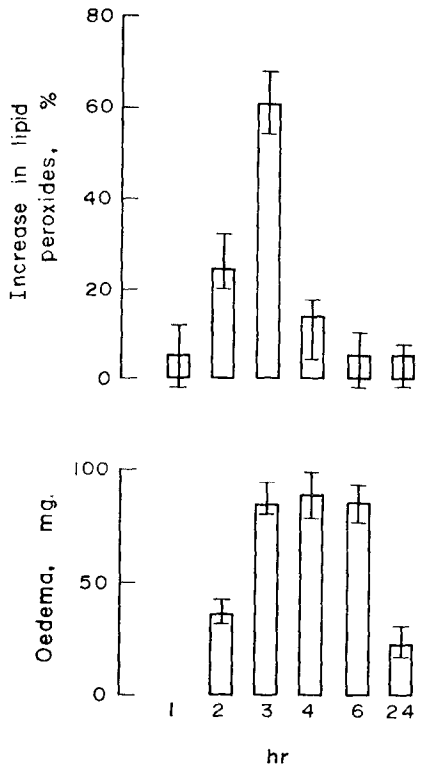

FIG. 1. Relationship between extent oedema and *in vitro* lipid peroxidation in liver homogenates of mice. The bars with respect to lipid peroxides represent the percentage increase over the zero time controls.

did not receive the drug or carrageenin. In animals which received carrageenin subsequent to phenylbutazone, both the development of oedema and the output of liver lipid peroxides were significantly inhibited as compared to mice which only received carrageenin. Curcumin did not have any action on the output of liver lipid peroxides in mice receiving the drug orally. However, in mice which received curcumin first and carrageenin 1 hr later, both the development of oedema and lipid peroxide production were promptly arrested. In contrast to the action of these two anti-inflammatory drugs, rutin neither protected the animals from oedema formation nor did it prevent the excess output of lipid peroxides by liver of inflammed mice.

It would be apparent from the above results that carrageenin triggered some as yet uncharacterized metabolic reaction in the liver. As a result of this metabolic disturbance, the *in vitro* output of lipid peroxides in liver was increased within 3 hr of administering carrageenin. Since damage of lysosomes is implied in the increased output of liver lipid peroxides,[5] it is likely that carrageenin elicits some local reaction in the paw which is transmitted to the liver in which, in turn, activation of lysosomes takes place possibly as a defensive measure.

The effect of curcumin and phenylbutazone as compared to that of rutin on *in vitro* lipid peroxidation is brought out by results summarized in Table 3. Rutin is a powerful anti-oxidant of *in vitro* lipid peroxide production but has no such property *in vivo* as shown in an earlier study[6] as well as confirmed in the present report. Curcumin inhibits *in vitro* lipid peroxidation but has no such effect *in vivo*; nonetheless it is able to protect mice from the inflammatory reaction elicited by carrageenin and the consequent increased output of liver lipid peroxides. Phenylbutazone, in contrast, had a stimulatory action on *in vitro* lipid peroxidation and a partial inhibitory action *in vivo* and prevented both inflammation and increased liver peroxide output. The antiflammatory effect of curcumin and phenylbutazone is thus not correlated with their action *in vitro* on lipid peroxide formation.

TABLE 2. EFFECT OF ADMINISTRATION OF PHENYLBUTAZONE, CURCUMIN AND RUTIN 1 hr PRIOR TO OEDEMA PRODUCTION BY CARRAGEENIN IN MICE

Treatment	Oedema (mg)	Lipid peroxide formation	Treated/Control
None	0	12·7 ± 0·37	1·00
Phenylbutazone	0	9·4 ± 0·28	0·74
Curcumin	0	12·0 ± 0·32	0·94
Rutin	0	12·2 ± 0·40	0·96
Carrageenin	84	21·3 ± 0·42	1·68
Carrageenin + Phenylbutazone	52	10·1 ± 0·17	0·79
Carrageenin + Curcumin	53	12·8 ± 0·19	1·00
Carrageenin + Rutin	74	20·7 ± 0·41	1·63

Animals were sacrificed 3 hr after administration of carrageenin.

Fresh homogenates of liver showed the presence of only 0·30 μmoles of malonyldialdehyde per mg of tissue.

One hr prior to injecting carrageenin, 80 mg/kg body weight of phenylbutazone was given intra peritoneally or 500 mg/kg body weight of curcumin or 300 mg/kg body weight of rutin was given orally.

Curcumin: Diferuloyl methane.

Phenylbutazone: 4-Butyl-1,2-Diphenyl pyrazolidine-3,5-dione. Rutin: Rhamnoside of hesperidin.

TABLE 3. EFFECT OF PHENYLBUTAZONE, CURCUMIN AND RUTIN ON LIPID PEROXIDE FORMATION BY FRESH HOMOGENATES OF MOUSE LIVER

	Additions	Lipid* peroxide formation
Experiment I	None	15·0
	Phenylbutazone $3{\cdot}42 \times 10^{-5}$ M	33·0
	Phenylbutazone $1{\cdot}71 \times 10^{-4}$ M	38·6
	Phenylbutazone $3{\cdot}42 \times 10^{-3}$ M	41·0
Experiment II	None	13·9
	Curcumin $1{\cdot}36 \times 10^{-4}$ M	8·2
	Curcumin $5{\cdot}44 \times 10^{-4}$ M	5·5
	Curcumin $5{\cdot}44 \times 10^{-3}$ M	0
Experiment III	None	12·9
	Rutin 1×10^{-5} M	3·3
	Rutin 5×10^{-5} M	0

* μmoles malonyldialdehyde 1 mg/3 hr.

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Determination and physiological disposition of dimethyltryptamine and diethyltryptamine in rat brain, liver and plasma

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DIMETHYLTRYPTAMINE (DMT) is the *N*-methylated analog of tryptamine. The compound occurs in various plants,[1] can be formed in rabbit lung and chicken brain,[2,3] and has been implicated in the pathogenesis of schizophrenia.[4,5] Diethyltryptamine (DET) does not seem to occur naturally. Both compounds are hallucinogenic in man and are occasionally abused for this reason.[6,7] In animals, they produce "abnormal" behavior and their effects on operant behavior have been carefully studied.[8–11]

Conclusions on the site and mode of action of DMT and DET and comparisons of the behavioral effects of these compounds with those produced by other psychoactive compounds have remained speculations so far, since they had to be based on doses injected. However, the injected dose is no indication of actual brain levels, since these levels are the result of a variety of factors including absorption, distribution, metabolism, excretion and penetration through the blood–brain barrier.[12] For this reason we developed a rapid assay procedure for the determination of injected DMT and DET in biological tissues and fluids, and we determined the concentrations of both compounds in rat brain, liver and plasma as a function of time and dose.

The assay procedure is based on the native fluorescence of DMT and DET after extraction of the compounds from biological tissues or fluids. The tissues (approximately 1–2 g) were homogenized in 3 ml of 1 N HCl. To the homogenates or plasma (1·0 ml plasma and 3 ml of 1 N HCl) 1·5 ml of 5 N NaOH and 30 ml toluene (ACS certified) were added. After shaking and centrifugating for 10 min, an aliquot of 25 ml toluene was removed and shaken with 1·5 ml of 0·1 N HCl for 10 min. After centrifugation for 10 min, 1 ml of 0·1 N HCl was combined with 1 ml of 0·1 M sodium borate buffer (pH 9·1), mixed, and then read in an Aminco-Bowman spectrofluorophotometer at 360 mμ (activation 280 mμ). Readings of extracts from plasma, brain and liver obtained from untreated animals were negligible. The sensitivity of the procedure is approximately 0·07 μg per ml or per g of sample and the recovery is approximately 80 per cent. The variability of the procedure showed a standard deviation of approximately 10 per cent of the mean.

To verify the assay procedure, tissue extracts from animals injected with DMT or DET were compared with the pure chemicals. A total spectral scan, the Brodie distribution test,[13] and thin-layer chromatography (chloroform–methanol–acetic acid, 75 : 30 : 5; ethanol–NH_3–H_2O, 8 : 2 : 1; and benzene–dioxane–NH_3, 50 : 45 : 5) showed that the assay procedure measured only DMT and DET.

DMT and DET were rapidly absorbed from the intraperitoneal cavity and quickly distributed through plasma, liver and brain (Table 1). Metabolism was also fast and most of the compounds had disappeared from brain, liver and plasma within 30 min, except DET in brain, which could still be detected at 60 min. The brain/plasma ratios of DMT and DET of 5·4 and 10·5 seem to indicate that the compounds cross the blood–brain barrier easily and are perhaps accumulated by an active transport mechanism.