Table II. Influence of various anti-inflammatory compounds on the production of lactic acid and utilization of glucose in the chick embryo culture

Compounds	Concentration (μmoles)	Lactic acid (μmoles)	Glucose (μmoles
Phenylbutazone	2500	5.5	n.d.
none	—	1.7	n.d.
Mefenamic acid	500	3.8	n.d.
none	—	2.0	n.d.
1,3-dicyclohexyl-barbituric acid	250	4.6	1.8
none	—	1.7	5.0
1-cyclohexyl-5,5-diallyl-barbituric acid	250	4.2	2.0
none	—	2.0	4.8
3-methyl-5-benzoyl-aminoiso-thiazole-4-carboxy-p-chloro-phenylamine	12	3.7	2.5
none	—	1.7	6.1
3-methyl-5-benzoyl-amino-isothiazole-4-carboxy-p-ethoxy-phenylamine	12	5.2	1.7
none	—	1.5	5.5

In all experiments the chick embryo tissue cultures were incubated for 2 days at 37 °C. The doses of the drugs used were those giving the maximal effect. n.d. = not done.

large doses of salicylate produce a profound decrease in glucose concentration and accumulation of lactic acid in the brains of young mice. Whereas in the blood the deficiency of glucose is immediately compensated by glycogenolysis.

Recently also DOERY and HIRSH[5] investigated the effect of the therapeutic levels of aspirin and salicylate on the glycolysis of platelets. They suggested that the drugs have divergent action: aspirin caused a fall in glucose concentration in the medium whereas salicylate was without effect. We believe that the lack of activity of salicylate in their experiments may be due to the application of too low concentration of the drug since salicylate is almost twice less active than aspirin e.g. in the test of stabilization of human erythrocytes[11].

We suggest that the determination of lactic acid production and glucose uptake in tissue culture may be useful for the preliminary screening of drugs for the anti-inflammatory activity.

Zusammenfassung. In der Kultur embryonalen Hühnchengewebes kommt es unter Einfluss nichtsteroider Antiphlogistica (Aspirin, Indomethacin, Mefenamicsäure) zu einer Steigerung des Glukoseverbrauchs bei gleichzeitiger Vermehrung der Milchsäureerzeugung. Der Milchsäureverbrauch verläuft in zwei Phasen, wobei der erste Gipfel bei geringsten Konzentrationen des Arzneimittels auftritt.

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Effects of Nicotine and Arousal on the Monkey Electroencephalogram

The effects of intravenous administration of intermittent doses of nicotine and oral doses of caffeine and D-amphetamine have been investigated on the EEG of the conscious squirrel monkey. The intermittent i.v. injection for nicotine was considered to closely simulate the uptake of nicotine during smoking, whilst the oral route for caffeine and amphetamine represented the normal route of administration for these drugs.

The results reported suggest that smoking doses of nicotine may produce an EEG state close to that obtained in normal alert situations; this is in contrast to that induced by the other centrally acting stimulants D-amphetamine and caffeine, suggesting an abnormal state after administration of these drugs.

Method. Twelve adult squirrel monkeys of either sex were used in this study. Platinum electrodes were implant-

Table I. Differences from control values in total EEG activity of the squirrel monkey EEG (left frontal-left occipital) following different drug treatments

Treatment	No. of animals	No. of experiments	Total EEG activity % change from control	Significant difference from arousal state (p)
Arousal	11	35	−41.1	
Saline	7	9	− 4.3	< 0.01
Nicotine (1 μg/kg/min i.v.)	10	11	−11.9	< 0.01
Nicotine (5 μg/kg/min i.v.)	6	8	−37.1	
D-amphetamine (0.1 mg/kg p.o.)	5	5	−17.6	< 0.01
D-amphetamine (0.5 mg/kg p.o.)	5	5	−50.8	
Caffeine (50 mg/kg p.o.)	5	8	−34.7	

Total EEG activity for nicotine was determined in the last 9 min of a 20 min intermittent i.v. injection period, and for caffeine and D-amphetamine during the 26–35 min following oral dosing.

ed on to the surface of the cortex under surgical anaesthesia. Electrodes were positioned bilaterally on the frontal, parietal and occipital cortex and were attached to a common connector on the surface of the skull. At the same time a cannula was implanted into the external jugular vein and connected subcutaneously to a ball valve cemented to the skull: this allowed i.v. injection in the conscious animal. After recovery from surgery animals were trained daily over a period of 4–8 weeks to accept the recording procedure. For recording the EEG animals were placed in a restraining chair in a sound-attenuated box and provided with adequate lighting and ventilation. EEG's were displayed on an Elema-Schönander Mingograf EEG recorder. One channel of the EEG (left frontal-left occipital) was submitted to a frequency analysis, using a series of narrow band filters tuned to centre frequencies in the range 2–30 Hz. The analyzer is similar to that described elsewhere[1]. The electrical activity in each frequency band over successive ten second periods was integrated and logged directly on to eight hole punch tape for subsequent processing using an Elliott 903 computer. Frequency analysis results were expressed as percentage change from control values in order to reduce inter-animal and inter-experiment variations to a minimum.

Before recording a pre-dose control EEG for a particular day, animals were allowed 20 min to acclimatize to the recording environment. It was found that after this period of time all trained animals would be in a similar behavioural state with a normal resting EEG. Nicotine, as nicotine hydrogen tartrate, was administered by intermittent intravenous injection at 1-minute intervals over a 20 min period at dose rates of 1 or 5 μg/kg/min. These doses were within the range for accepted smoking doses of nicotine[2]. D-Amphetamine (0.1 mg/kg and 0.5 mg/kg) and caffeine (50 mg/kg) were administered orally in 1 ml distilled water. The dose for caffeine was selected as that which was sufficient to cause a desynchronization (flattening) in the EEG 30 min post oral dosing. Those for amphetamine were selected as being within the normal therapeutic range. In practice the high dose of amphetamine was found to be sufficient to cause desynchronization of the EEG 30 min post oral dosing.

Frequency analysis results following i.v. administration of nicotine were determined over the last 9 min of the 20 min nicotine administration period, and following caffeine or D-amphetamine over the 9 min period 26–35 min post oral dosing. Frequency analysis results for the control were determined in the last 9 min of a 20 min period immediately following the 20 min acclimatization period and immediately preceding drug administration. Arousal of an animal was produced by presenting continually, novel visual and audio stimuli throughout the 9 min period to give the frequency analysis results for the arousal situation. The total EEG activity (defined as the integral of a rectified EEG signal) over a 10 sec period is related to the arousal or resting state of an animal over that period. That is, a desynchronized or flat EEG as might be obtained in an arousal situation would give less total EEG activity than an EEG representing a resting state.

Results. Examination of the EEG record following administration of high doses of all the drugs showed a pattern very similar to the EEG induced by alerting the

Table II. Comparison of the differences from control values in activity in different frequency bands of the squirrel monkey EEG (left frontal-left occipital) following high doses of D-amphetamine, caffeine and nicotine with those following behavioural arousal. These high doses were sufficient to cause a change in EEG total activity insignificantly different from that following behavioural arousal (see Table I).

Treatment	No. of animals	No. of experiments	Band centre frequencies (Hz)													No. of frequency bands in which % change in activity differed significantly from the alert
			3	4	5	6	7	8	9	10	11	12	14	16	18	20	
			Change of activity from control (%)														
Arousal	11	35	–33.8	–38.5	–43.2	–44.2	–46.6	–47.8	–56.2	–55.6	–56.1	–51.9	–31.8	–19.9	–15.4	–10.8	
Nicotine (5 μg/kg/min i.v.)	6	8	–39.1	–45.3	–51.9	–56.0	–54.8	–57.3	–57.0	–50.8	–49.1	–38.9	–19.4	–13.9	–11.4	–16.4	None
D-amphetamine (0.5 mg/kg p.o.)	5	5	–64.2[b]	–73.5[b]	–75.6[b]	–70.8[b]	–66.7[b]	–63.8[a]	–59.2	–51.8	–39.4	–26.7[a]	– 9.9[a]	–10.8	–13.4	–18.8	8
Caffeine (50mg/kg p. o.)	5	8	–35.6	–42.1	–44.8	–40.4	–36.0	–24.5[b]	–21.4[b]	–11.1[b]	–19.4[b]	–32.8	–42.9	–51.3[b]	–47.2[b]	–48.9[b]	7

Significance of difference from arousal atate [a] $P < 0.05$, [b] $P < 0.01$.

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animal. Comparison of the percentage change from the control of the total EEG activity following administration of the higher dose of all 3 drugs (Table I) showed decreases which are not significantly different from those found by alerting the animal. If the drug-induced EEG patterns obtained at these dose levels did not differ from those in an arousal situation then the percentage change in the different frequency bands when compared with the control values would not have differed from those found by alerting the animal. In Table II it can be seen that the percentage changes in a number of EEG frequency bands following D-amphetamine (0.5 mg/kg) and caffeine (50 mg/kg) administration were significantly different from those of an alert state. The EEG following nicotine administration (5 µg/kg/min) did not differ significantly in percentage change in any frequency band from the alert state.

Discussion. These results raise a number of interesting questions. The possibility that nicotine produces an EEG arousal state which is closer to a normal arousal than either caffeine or D-amphetamine is in accord with present knowledge of drug interactions on the central nervous system. Nicotine type cholinergic neurons exist[3] and there is evidence for their presence in the central nervous system[4]. The results of KAWAMURA and DOMINO[5] suggest that nicotine may act primarily in the mid brain reticular formation. This structure plays an important role in the arousal response[6], and nicotine may act primarily by stimulating the normal arousal pathways. No such receptors or mechanisms have been suggested for amphetamine or caffeine. The suggestion that nicotine induces a 'normal' arousal state whilst other centrally acting stimulants do not is well fitted by these results. It could be however, that the effect is general for central nervous system stimulants, but dose-related, though the results to date do not suggest this. It is fully realised that the results are based on EEG's from only one area of the cortex using relatively few dose levels. It is intended to conduct more studies along lines to test the validity of this hypothesis further.

Résumé. Les auteurs ont établi qu'après des doses de nicotine, telles qu'on en absorbe en fumant une cigarette, le tracé de l'EEG ressemble plus à celui qui se présente après une réaction d'alerte qu'après l'administration d'amphétamine ou de caféine.

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The Effect of Intoxication Induced in Rat Liver by Carbon Tetrachloride, Ethionine and White Phosphorus on the Level of Microsomal Cytochromes b_5 and P_{-450}

The analysis of the microsomal cytochromes b_5 and P_{-450} may represent an useful tool in the study of the pathogenesis of hepatic fat accumulation induced by administration of carbon tetrachloride (CCl_4), ethionine or white phosphorus.

The action of CCl_4, which involves a lipoperoxidative mechanism by the liberation of $CCl_3^{\bullet}$ free radicals, appears to be primarily concerned with the membranes of the endoplasmic reticulum. This lesion is accompanyed by an early accumulation of triglycerides, followed by a mitochondrial injury (ref.[1] for review). A decrease of cytochrome P_{-450} has also been reported by SMUCKLER[2] in the liver microsomal fraction of rats treated orally for 2 h with CCl_4. Ethionine is supposed to interfere with ATP synthesis, through the formation of S-adenosyl ethionine, a competitive analog of S-adenosyl methionine[3]. As a consequence, there is a reduced availability of ATP and a decrease in the intracellular concentration of K^+[4]. The steatogenic effect of white phosphorus would also imply a lipoperoxidative mechanism similar to that of CCl_4[5].

The aim of the present work is to establish whether the morphological damage induced by CCl_4, ethionine or white phosphorus poisoning in liver is accompanied by changes in the level of the microsomal respiratory pigments, which may be correlated to a lipoperoxidative mechanism. Moreover, since in vitro experiments[6,7] have shown the conversion of cytochrome P_{-450} into a solubilized form, called cytochrome P_{-420}, owing to the modification of the lipoproteic shell around the haemoprotein molecule, we attempted to establish whether a similar modification could happen in vivo by the lipoperoxidative action of CCl_4.

The results obtained indicate that the pathogenic mechanisms of the steatogenic agents employed are different as far as the spectral determination of the content of microsomal pigments is concerned. A preliminary account of this work has been presented[8].

Materials and methods. Female albino rats of the Wistar strain, weighing 200–250 g, fasted for 16 h, were treated with CCl_4 (0.25 ml/100 g body weight, i.p. or by gastric intubation). with ethionine (100 mg/100 g body weight, i.p.) or white phosphorus (0.75 mg/100 g body weight, by gastric intubation), and sacrified according to the scheme shown in the Table. At the times of treatment chosen for the different drugs a pronounced hepatic fat accumulation and reduction in the level of circulating lipoproteins is observed[9–11]. For CCl_4 intoxication assays have also been performed at the early stages of treatment.

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