Acetazolamide (Diamox) was obtained from Lederle Laboratories (India). It was dissolved in ethanol to give a 2·5 mM solution. 0·4 and 1·0 ml aliquots of this solution were included in 100 ml of the saline medium, thereby giving a final concentration of 10 and 25 μM of acetazolamide per litre. The same amounts of ethanol were added to the control flasks also.

The results in Table 1 show that acetazolamide lowers the respiratory rate of the slices at the level of 25 μM/1. The inhibition is seen to be much more pronounced in the calcium-free medium than in the calcium-containing medium. This may imply that calcium ions play a role in chloride ion transport or it may simply mean that calcium ions interfere with the inhibitory action of acetazolamide.

Depletion of calcium ions from the incubating medium enhances the respiratory rate (columns 1 and 4, Table 1). But this effect is not seen when acetazolamide is present in the medium (columns 3 and 6). This is similar to a report by Davis and Dettbarn[7] that the depolarising action of Ca^{++} depletion in frog nerve is inhibited by several drugs which act on the acetyl choline system.

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Inability of 1-amino cyclopentane carboxylic acid to prevent in the liver the induction of glutamic-pyruvic transaminase by hydrocortisone

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IN A recent publication,[1] we have reported on the selective nature of the damage caused by 1-amino cyclopentane-1-carboxylic acid (ACPC) to the acinar cells of the pancreas.

Since ACPC was responsible for a loss in the biosynthesis of proteolytic enzymes in the pancreas,[1] it was interesting to find out whether the compound would also affect other enzymatic systems in the liver.

We wish to report the results obtained on the RNA content and glutamic-pyruvic transaminase activity of the liver after ACPC and hydrocortisone treatment, which is known to cause an increase of glutamic-pyruvic transaminase activity.[2]

METHODS

ACPC was synthesized in our own laboratory, and hydrocortisone acetate was a commercial sterile suspension obtained from Merck Sharp and Dohme of Canada, Ltd.

Twenty-four male albino rats of the Wistar strain, with body weights ranging from 147 to 172 g, were divided into four equal groups. The mean body weight of each group was 160 g. All animals were isolated in individual cages and maintained on Purina laboratory chow and tap water *ad libitum*.

Group H received 7·5 mg hydrocortisone acetate daily for 7 days by subcutaneous injection. Group A received daily i.p. injections of ACPC in amounts calculated so that each animal received a total amount of 350 mg/kg body weight after 7 days. Group HA received a combination of the two treatments just descrived. Group N served as control. All the animals were sacrificed by guillotine, their livers quickly excised and homogenized in a Waring Blendor with a suitable quantity of ice-cold

distilled water, and the homogenates were stored at −15°. An aliquot of the liver was also homogenized in a Potter apparatus in 10% trichloroacetic acid, and set aside for RNA content determinations. Within 4 days, the frozen homogenates were all assayed for glutamic-pyruvic transaminase activity, by a modification of the colorimetric transaminase determination described by Tonhazy *et al*[3]. The determination of the RNA of the liver was made by the classical method of Dische and Schwartz.[4]

RESULTS AND DISCUSSION

Table 1 shows that the administration of a highly toxic dose of ACPC (350 mg/kg) has no effect on the glutamic-pyruvic transaminase activity. At this dosage ACPC is able to destroy exocrine pancreas

TABLE 1. EFFECT OF HYDROCORTISONE AND ACPC ON THE GLUTAMIC-PYRUVIC TRANSAMINASE ACTIVITY AND ON THE RNA CONTENT OF RAT LIVER

Treatment	Glutamic-pyruvic transaminase activity (mmoles substrate utilized h/g liver wet wt.)	RNA content (mg/100g liver wet wt.)
Controls	1·02 ± 0·12 (6)*	50·19 ± 6·4 (6)*
ACPC (50 mg/kg/day for 7 days)	1·05 ± 0·13 (6)	48·18 ± 4·7 (5)
Hydrocortisone (7·5 mg/day for 7 days)	2·80 ± 0·11 (5)	36·52 ± 6·1 (5)
Hydrocortisone + ACPC (Combination of both treatments	2·46 ± 0·21 (6)	50·43 ± 5·9 (5)

* Mean ± standard error (number of animals).

in 7 days in rats and will kill all animals within 12 days. The results also show that even in the presence of ACPC, the liver responds to the enzymatic induction produced by the hydrocortisone treatment. Statistically, all the results in this table are highly significant. The administration of ACPC did not affect significantly the RNA content of the liver. It is also shown that chronic treatment with hydrocortisone results in a decrease in the RNA content of the liver. These data, which are not statistically significant, are however in accord with the results reported by Lowe and Rand,[5] who found similar changes in the cytoplasmic RNA of the liver after cortisone administration. Our results show that combination of ACPC and hydrocortisone treatments has no effect on the RNA content.

Glutamic-pyruvic transaminase, a key enzyme in the protein metabolism in liver, has been shown to increase after treatment with glucocorticosteroids.[2] Any modification of the protein metabolism in the liver would also be reflected by changes in the activity of glutamic-pyruvic transaminase. One can then assume that any cytotoxic drug liable to interfere with liver enzyme biosynthesis would have an effect on this enzyme. It is therefore significant that ACPC, which is so detrimental to exocrine pancreas structure and function,[1] produces no observable effect on liver function, as assayed by the glutamic-pyruvic transaminase test. We had found that ACPC *in vitro* has no effect on glutamic-pyruvic and glutamic-oxaloacetic transaminases,[6] and we later found out that it was also ineffective, when added *in vitro*, on the proteolytic enzymes of the pancreas. Moreover, when the activity of this transaminase is induced by glucocorticoid treatment, it does so irrespective of concomitant ACPC administration at highly toxic dose. The absence of any significant change in the RNA content of the liver, in the presence of ACPC, is in line with the above observations and substantiates our claim that ACPC does not impair the liver in one of its main functions. Its direct effect is confined to the exocrine function of the pancreas where it causes structural damage.

This is a case where a compound can impair the biosynthesis of an enzyme in a specific gland—the pancreas—but is ineffective in altering the biosynthesis of another type of enzyme related to protein metabolism in another organ—the liver.

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On the relationship between lipid solubility and microsomal metabolism of drugs

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GAUDETTE and Brodie,[1] in a study of 28 drugs and natural products containing methyl or other alkyl groups, found that only those compounds which were highly lipid soluble were oxidatively dealkylated by rabbit liver microsomes *in vitro*. They suggested that either the enzymes involved were protected by a lipid barrier which only fat-soluble substances could penetrate or that only nonpolar compounds could intereact with their active sites. McMahon and Easton[2,3] also observed a correlation between lipid solubility and rate of liver microsomal demethylation of other drugs. They concluded that the ipid solubility of a substrate was rate-limiting for this process, and accepted the suggestion of Gaudette and Brodie concerning the localization of the enzymes involved behind a lipid barrier.

The results of these three studies have been emphasized by reviewers of the subject of drug metabolism, who have attempted to generalize concerning the nature and function of the microsomal drug-metabolizi- g enzymes. Thus Gillette[4,5] and Brodie and Maickel[6] concluded that all foreign compounds that are oxidized by liver microsomal enzymes are nonpolar and that the oxidation products are less lipid soluble than the parent compounds. They considered the function of this process as increasing urinary excretion of foreign compounds by decreasing tubular reabsorption, a process that depends on solubility in the lipoidal membranes of the kidney tubules.

Exceptions can be found to many generalizations, including the one under consideration here, and the examination of these exceptions may lead to clarification and extension of the concepts involved. We wish to present here some data that are not compatible with the generalization that there is a correlation between rate of metabolism and degree of lipid solubility of the substrates of microsomal oxidative enzymes. These exceptions were encountered during studies of the metabolism of S-methyl compounds and of certain antimetabolites[7, 8].

The demethylation of a variety of substrates was followed by determining the amount of formaldehyde formed during incubation with microsomes plus soluble fraction from mouse or rat liver homogenates. Incubation conditions and analytical procedures have been described previously[7, 9] and are summarized in Table 1. Lipid solubility was estimated by determining at 25° the partition of each substrate between chloroform and 0·1 M phosphate buffer, pH 7·4 and is expressed as the per cent present in the chloroform phase after equilibration.

The representative data presented in Table 1 clearly indicate that there is no obvious correlation between the rate of demethylation of the substrates studied and their solubility in chloroform. Outstanding are the high rates of demethylation of the water-soluble compounds, puromycin aminonucleoside and 1-methylguanosine.