(30 sec). One plate of cells was used to measure the $^{24}Na^+$ content at zero time and several plates were used to determine the $^{24}Na^+$ left in the cells after various time intervals in radioactive-free solution, a separate plate of cells being used for each time interval. All experiments were done at 21° C.

The Na^+ exchanges with a $t\frac{1}{2}$ of about 3 min, with a mean value of about 4·5 p-mole/cm² per sec. This is similar to other cells (Burrows & Lamb, 1962). Ouabain (10^{-3}M) for 30 sec reduces the Na^+ efflux to about 40% of its previous value. Removal of KCl from Krebs (KCl < 0·3 mM) reduces the Na^+ efflux to about 50% within 30 sec. These results indicate that the Na^+ efflux is largely active, coupled to K^+ and sensitive to ouabain.

Further experiments showed that after 4 hr in the presence of ouabain, the Na^+ efflux was slightly greater than the control. Removal of K^+ (in the presence of ouabain) reduced the Na^+ efflux to about 60%. Neither the immediate application (30 sec) nor the prolonged treatment (4 hr) of ouabain appears to affect the Na^+ influx, measured over 30 sec. The cellular content of Na^+ in cells treated with ouabain increased from 8 mmoles/l. intracellular water to about 35 mmoles/l. intracellular water in the first 2 hr, and then remains about this level for the next 6 hr. If, after 4 hr treatment with ouabain, the cells are transferred to a K^+-free Krebs solution containing ouabain for a further 2 hr, the Na^+ content rises to just over 50 mmoles/l. intracellular water.

We interpret these results as showing the existence of an ouabain insensitive K^+ coupled Na^+ pump in L cells. Recent experiments suggest that either an increase or decrease in the cellular levels of Na^+ or K^+ respectively control the amount of active transport in the presence of ouabain.

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The uptake of tetracycline by human blood cells

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The native fluorescence of tetracycline in blue light has been used to determine its localization in human blood cells. Samples of venous blood were incubated for one hour at 37° C with concentrations of tetracycline hydrochloride between 10 μg/ml. and 1 mg/ml. Unstained smears of the cell suspensions were then examined by phase-contrast and fluorescence microscopy.

The intense yellow fluorescence characteristic of tetracycline was found only in the leucocytes; the erythrocytes appeared uniformly dark. Measurement of the cellular fluorescence revealed a dose-dependency over the concentration range examined. Uptake of tetracycline appeared to depend also on incubation time;

fluorescence was not detectable in smears made immediately after exposure to the drug. The addition of ascorbic acid as a buffer for tetracycline did not affect uptake into leucocytes, but enhanced the stability of the fluorophore.

Since cellular fluorescence was detectable at drug concentrations of 10 μg/ml. (a serum level attainable therapeutically) blood films from patients receiving tetracycline were examined. The results obtained were essentially similar to the *in vitro* observations.

In both lymphocytes and polymorphonuclear leucocytes the nucleus exhibited more intense fluorescence than the cytoplasm. This is contrary to the findings of du Buy & Showacre (1961) and Zuckerman, Baker & Dunkley (1968) that tetracycline is bound specifically to mitochondria in mammalian cells. The enhanced fluorescence in the nucleus may be a result of combination of the drug with DNA as described by Kohn (1961).

Further investigations are needed to elucidate the clinical importance of these results.

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A new metabolite of carbon tetrachloride

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The possibility of trichloromethyl radicals arising from homolytic cleavage of carbon tetrachloride *in vivo* was put forward by Butler (1961), and a role in hepatotoxicity for such radicals has been discussed (Slater, 1966). Gas-liquid chromatography with electron capture detection has provided a sensitive means of detecting $Cl_3C.CCl_3$ (Fowler, 1969). Traces of this dimer, which caused liver damage in sheep (Fowler, 1969) were detected in tissues of rabbits to which carbon tetrachloride had been administered.

Samples of liver, kidney, fat, muscle and bile were taken from rabbits 0, 6, 24 and 48 hr after administration of carbon tetrachloride (1 ml./kg) by stomach tube (20% v/v in olive oil). After extraction by heptane partition, carbon tetrachloride, chloroform and $Cl_3C.CCl_3$ were separated on an SE–30/Celite column. Identification of $Cl_3C.CCl_3$ was supported by comparison of retention times with standards on three columns: SE–30/Celite; SE–30/PEG 20M/firebrick and di(2-ethylhexyl) sebacate/chromosorb G.

Maximum concentrations (with standard deviations) of CCl_4 were in fat (6 hr: 787 ± 289 μg/g); of $CHCl_3$, in liver (6 hr: $4{\cdot}9 \pm 1{\cdot}5$ μg/g); and of the dimer, $Cl_3C.CCl_3$, in fat (24 hr: $16{\cdot}5 \pm 1{\cdot}6$ ng/g).

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