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THE UPTAKE AND LOCALIZATION OF TETRACYCLINE IN HUMAN BLOOD CELLS

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SUMMARY.—The uptake of tetracycline by human blood cells has been investigated by means of fluorescence microscopy. The intense yellow fluorescence characteristic of the drug was found only in the leucocytes; the erythrocytes appeared uniformly dark. In both lymphocytes and polymorphonuclear leucocytes the nucleus exhibited more intense fluorescence than the cytoplasm. The importance of these results is discussed.

THE autofluorescence of tetracycline has enabled studies of its localization in living cells to be made. Du Buy and Showacre (1961) using mouse tissues and Zuckerman, Baker and Dunkley (1968) using human foetal liver cells have provided evidence that the antibiotic is selectively bound to the mitochondria in mammalian cells. However, Dressler and Mach (1965) found increased fluorescence in the nuclei of chick erythrocytes incubated with high concentrations of tetracycline *in vitro*. In an attempt to clarify this matter we have examined the penetration of the antibiotic into normal human blood cells both *in vivo* and *in vitro*.

MATERIALS AND METHODS

Freshly-drawn venous blood was collected into glass tubes containing heparin (5 u./ml. blood) or EDTA (final concentration 0·1 per cent). The choice of anticoagulant was not critical. Samples (1·0 ml.) were incubated at 37° for 1 hr with 0·1 ml. 0·9 per cent NaCl or with tetracycline hydrochloride (Lederle) dissolved in the saline at various concentrations between 10 μg./ml. and 1 mg./ml. Blood films were made immediately after adding the drug and after the incubation period when the cells had been washed twice with drug-free autologous plasma. The native fluorescence of tetracycline was used to determine its cellular localization in the unstained smears. Using a Zeiss Standard Universal G40–140 microscope with HBO 200 mercury light source maximum fluorescence was recorded with the UG 1 exciter filter (transmission 300–400 mμ) and barrier filter 50 (transmission above 500 mμ). The fluorescence characteristics of tetracycline in solution are an excitation maximum of 390 mμ and a fluorescence maximum of 515–520 mμ (Udenfriend, Duggan, Vasta and Brodie, 1957). Measurements of the plasma fluorescence in smears of unwashed cells were used to calibrate the microscope photometer (Zeiss MPM with type EM1 6256 B photomultiplier). For the study of cellular fluorescence the following procedure was used. The slide was first scrutinized under phase-contrast at a magnification of 1250 and an area containing erythrocytes and leucocytes located. The same area was then examined for fluorescence under violet light.

RESULTS AND DISCUSSION

The appearance of blood cells incubated with 200 μg. tetracycline/ml. for 1 hr is shown in Fig. 1. The intense fluorescence characteristic of the antibiotic is restricted to the leucocytes and the erythrocytes appear dark. In addition the

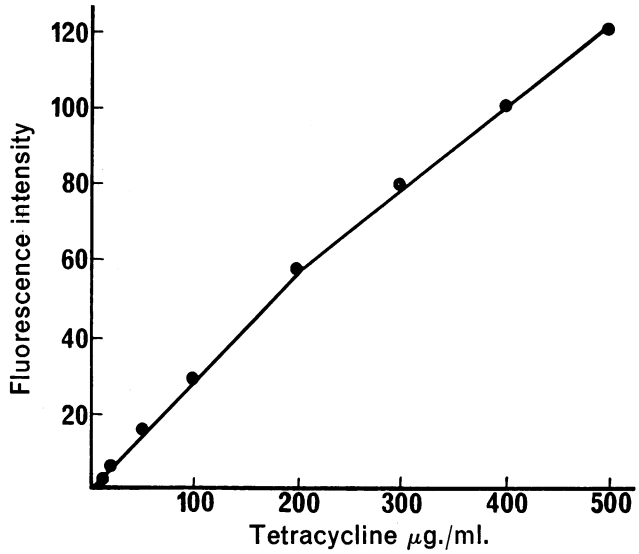


FIG. 2.—Calibration graph of fluorescence intensity against tetracycline concentration.

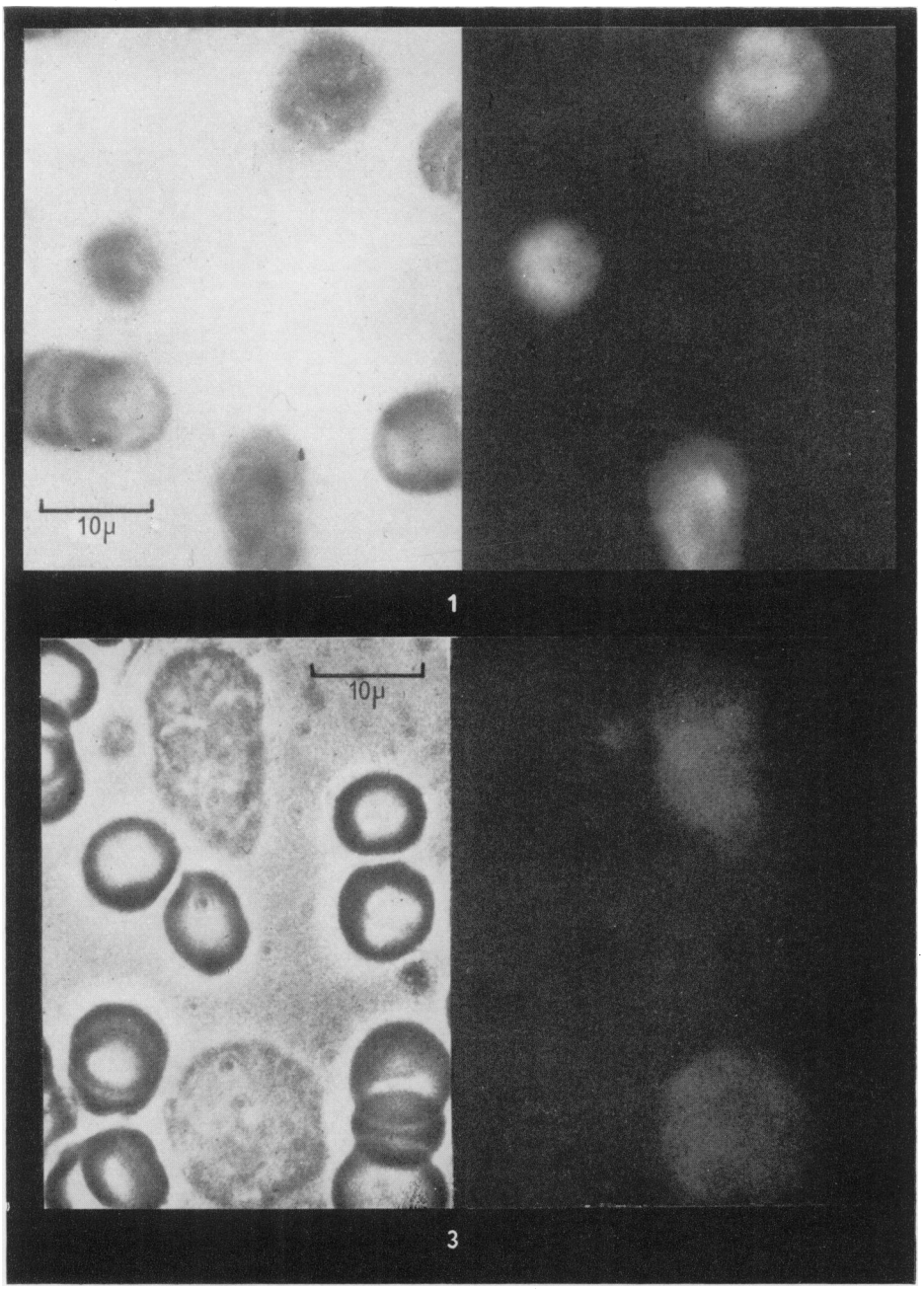
nucleus in the leucocyte is more highly fluorescent than the cytoplasm. This is readily apparent in the polymorphonuclear leucocytes. Since the same was observed in unwashed cells the lower cytoplasmic fluorescence is not due to diffusion of the antibiotic into the drug-free plasma during washing. The same observations were made following incubation with all the concentrations of tetracycline tested. However, in smears made immediately after mixing the blood with the drug and in saline controls there was no detectable fluorescence in the leucocytes. Addition of ascorbic acid to the drug (Achromycin, Lederle Laboratories) did not affect penetration of the antibiotic into the leucocyte but the stability of the fluorophor was enhanced. In smears stained with Leishman stain the morphology of cells incubated with tetracycline appeared normal.

The complete absence of fluorescence in the erythrocytes suggested that these cells do not take up tetracycline. However, the possibility existed that haemoglobin was completely quenching the native fluorescence of the drug. Accordingly the tetracycline concentration in samples of plasma and packed red cells was determined biochemically using the technique described by Kohn (1961*a*). The highly fluorescent complex formed through the interaction of tetracycline, calcium and barbitone was extracted into ethyl acetate thus permitting its separation from biological material. Tetracycline was found to be uniformly distributed between packed red cells and plasma over the concentration range 10–500 μg./ml.

EXPLANATION OF PLATES

FIG. 1.—Phase-contrast (on left) and fluorescence (on right) photomicrographs of blood cells exposed *in vitro* to 200 μg. tetracycline/ml. for 1 hr.

FIG. 3.—Phase-contrast (on left) and fluorescence (on right) photomicrographs of blood cells from patient receiving 250 mg. oxytetracycline 4 times a day. Plasma level was 3·8 μg./ml. Note more intense nuclear fluorescence.



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The failure to detect fluorescence microscopically is probably the result of colour quenching.

In order to determine the drug concentration within the leucocyte the fluorescence of individual cells was measured. With a magnification of 1250 the fluorescence intensity from the whole cell could be measured; suitable corrections for background were made in each case by measuring the fluorescence two cell-diameters removed from the leucocyte. The photometer was calibrated each time by measuring the fluorescence of the plasma in smears of unwashed cells. Fig. 2 shows the graph of fluorescence intensity of these patches of plasma against tetracycline concentration in μg./ml. The linearity of this calibration graph over the drug concentration range studied is apparent.

Using this procedure for calibrating the photometer the effects of temperature on the uptake of different concentrations of tetracycline into human leucocytes was investigated and the results are shown in the Table. It is apparent that there

TABLE.—*The Effects of Incubation Temperature on the Uptake of Different Concentrations of Tetracycline Hydrochloride (TC) into Normal Human Leucocytes*

Conc. of TC in medium μg./ml.	Conc. of TC in leucocytes in μg./ml. (mean ± S.E., $N = 20$)			
	Polymorphs		Lymphocytes	
	37°	20°	37°	20°
10	33·3±1·3	—	33·3±2·0	—
20	74·4±3·7	—	81·7±3·8	—
50	81·8±3·5	6·0±0·1	88·8±4·5	10·0±4·1
100	99·4±5·4	33·3±1·8	73·4±4·2	36·7±2·9
200	112·1±3·3	—	103·5±6·1	—
500	408·0±23·3	84·0±3·3	386·0±17·1	94·8±3·6

are no essential differences between the two types of leucocyte. At each drug concentration the fluorescence of 20 polymorphonuclear leucocytes and 20 lymphocytes was measured. At concentrations of tetracycline less than 100 μg./ml. the cellular fluorescence exceeds the medium fluorescence whereas at drug concentrations greater than 100 μg./ml. the converse is the case. The fact that at 20° the intracellular levels are much lower suggests that the concentrating mechanisms for tetracycline are temperature-dependent.

Since cellular fluorescence was measurable at an incubation concentration of 10 μg./ml.—a plasma level attainable therapeutically—blood from patients being treated with tetracyclines was examined. All 6 patients studied were receiving oxytetracycline orally for minor respiratory infections and plasma levels, measured by Kohn's method, (Kohn, 1961*a*), ranged from 1–5 μg./ml. Below 3 μg./ml. no fluorescence was apparent on microscopic examinations; above this concentration fluorescence was detectable in the leucocytes (Fig. 3) although reliable measurements of the cellular concentration could not be made. Since the plasma fluorescence was not detectable microscopically it would appear that leucocytes can concentrate tetracycline *in vivo* as well as *in vitro*. This point will be clarified when blood with higher plasma levels, preferably of tetracycline which is more highly fluorescent than oxytetracycline, can be examined.

Perhaps the most intriguing observation from these studies is that both *in vivo* and *in vitro* the nuclei in the lymphocytes and the polymorphonuclear leucocytes were more highly fluorescent (about 3 times) than the cytoplasm. There are a

number of possible explanations for this difference. The concentration of tetracycline in the nucleus may indeed be higher than that in the cytoplasm; or the cytoplasmic fluorescence may be quenched; or the nuclear fluorescence may be enhanced and there is some evidence for this. Kohn (1961*b*) found that tetracycline will bind to purified DNA in the presence of divalent metal ions and that the complex is about 4 times as fluorescent as tetracycline itself. If similar binding is occurring in the living cell this itself could account for the difference between the nucleus and the cytoplasm. To investigate this point we are repeating the work using tritiated tetracycline and detecting cellular localization by autoradiography. Whatever the mechanism of the nuclear binding, it is clear that tetracycline cannot be considered as a selective label for mitochondria.

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