added malate (or hydroxymalonate) citrate transport was inhibited until malate formed from pyruvate had reached sufficient concentration outside the mitochondria. On addition of bicarbonate plus malate or hydroxymalonate production of citrate was linear and accounted for 70% or more of the pyruvate uptake whereas metabolism of citrate through the tricarboxylate cycle was negligible; under these conditions oxaloacetate for citrate synthesis was largely formed from pyruvate through pyruvate carboxylase. In the absence of bicarbonate malate uptake was markedly increased and was sufficient to account for the oxaloacetate required for citrate synthesis. In fat-cells under physiological conditions (in the presence of bicarbonate), oxaloacetate for citrate synthesis would thus be expected to be formed largely from pyruvate through pyruvate carboxylase rather than from malate through malate dehydrogenase.

In parallel experiments on liver mitochondria prepared from fed rats, rates of citrate production were much smaller (in terms of mitochondrial protein), and in the presence of bicarbonate malate rather than pyruvate was the main source of oxaloacetate for citrate synthesis.

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Kinetics of 5-Hydroxytryptamine Exchange in Rabbit Blood Platelets and its Analysis by Digital Computer

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Digital-computer simulation of the effects of reserpine on the rate of 5-hydroxytryptamine uptake and distribution in rabbit blood platelets has been performed as part of a programme to assess the value of the technique in biochemical pharmacology.

Platelet-rich plasma was incubated either alone or with reserpine (50 $\mu\text{g}/\text{ml}$) for 20 min. Tritium-labelled 5-hydroxytryptamine was added, raising the plasma 5-hydroxytryptamine concentration to 4 $\mu\text{g}/\text{ml}$, and incubation was continued for additional periods up to 40 min. The platelets were spun down immediately at 0°C, washed and treated ultrasonically in 0.25M-sucrose. Soluble, mitochondrial and 5-hydroxytryptamine-storage-granule fractions were isolated from the ultrasonically treated material by centrifugation on sucrose density gradients. The specific radioactivity of the 5-hydroxytryptamine in each subcellular compartment relative to plasma at zero time was deter-

mined. Volumes of the compartments were found from the distribution of K^+ after storage of platelets at 0°C for 1 h and were approx. 0.2 ml/mg of platelet protein for soluble material (cytoplasmic compartment) and 0.05 ml/mg of protein for storage granules.

The concentrations of 5-hydroxytryptamine in the soluble and granule compartments were 8 and 80–100 $\mu\text{g}/\text{ml}$ respectively, remaining almost constant for the duration of the experiments. The exchange of 5-hydroxytryptamine between platelet contents and plasma proceeded rapidly; in the absence of reserpine the soluble fraction had a relative specific radioactivity of 0.11 at 10 min and 0.35 at 40 min and the granular material 0.08 and 0.22 at these times. Reserpine decreased these values to 0.09, 0.26 and 0.05, 0.17 respectively.

The complete kinetics of 5-hydroxytryptamine uptake and exchange were analysed on an ICL 1905 computer, with a program written by myself. The program combined in a novel way principles of simulation and curve-fitting described by Garfinkel (1968), Kibby (1969) and Powell (1965).

Models describing the transport of 5-hydroxytryptamine within the platelet were tested and increased in complexity, starting from the very simplest possible system, until an acceptable fit between the observations and predicted values was obtained. The final model contained zero-order and first-order transport mechanisms at both the platelet and granule membranes, and had four parameters which were estimated by the computer program. Reserpine was found to decrease the diffusion constant across the platelet membrane from 0.0045 ± 0.0017 to $0.0016 \pm 0.0006 \text{ min}^{-1}$ (mg of protein) $^{-1}$, and to decrease the zero-order constant for uptake into the platelet granule from 1.1 ± 0.7 to $0.7 \pm 0.2 \mu\text{g min}^{-1}$ (mg of protein) $^{-1}$. (Results are given as value $\pm$ S.E.M.)

These quantitative results agree with qualitative observations on the reserpine-induced depletion of biogenic-amine stores in platelets (Pletscher, Da Prada & Tranzer, 1968). They also appear to be the first numerical expression of the effect of reserpine on amine kinetics of the intact cell. The simulation technique leads to conclusions not hitherto obtainable without much greater experimentation, and by its flexibility should have a number of applications.

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