All injections were given i.p. The 34th batch was treated as control and given physiological saline injections. The last injection in all batches was followed by a tracer dose of 5.0 μc of I^{131} which was administered i.p. for the evaluation of thyroidal iodine uptake. The region of the lower jaw of the specimens containing thyroid follicles was cut out, blotted dry and its thyroidal iodine uptake was measured in well type scintillation counter.

Using the induced I^{131} uptake as an indicator of TSH activity, I found that this species exhibited seasonal cyclical variations in its thyrotropic potency. With normal photoperiodic conditions there were 2 peaks in TSH content alternating with phases of very low activity. From the Figure it can be seen that April and September gave peak values, January and July low values. The pattern of cyclic fluctuations was the same in group 2, exposed to continuous illumination, except that the peaks were encountered 2 months earlier than with group 1. In contrast to this, TSH level in the samples of group 3, which was kept under total darkness, was very low throughout the year and may be compared with the minimal potencies of the other 2 groups (Figure). The 34th batch which was treated as control showed very low uptake of I^{131}, due to the blocked indigenous secretion of TSH.

Under normal photoperiods the pattern of cyclic variations in TSH level resembles to some extent that of *P. fluviatilis*[17], where June and September samples showed an increase in TSH content but August samples were found to be inactive. Continuous illumination appears to be one of the important external factors responsible for the early activation of the pituitary without disturbing the basic plan of cyclic activity and thereby raising the peaks of thyrotropic content by 2 months in advance as compared to that kept under normal photoperiods. Total darkness seems to be an other external regulator which tends to abolish the inherent capacity of pituitary for cyclical activity[19].

Zusammenfassung. *Mystus vittatus* zeigt unter natürlichen Bedingungen jahreszeitliche Variationen des TSH Gehaltes der Hypophyse. Veränderung der Lichtverhältnisse kann diesen TSH Zyklus beeinflussen: Dauerlicht verschiebt die Phasen, Dauerdunkel unterdrückt die zyklischen Veränderungen auf einen geringen Aktivitätsgrad.

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Time Course of the Distribution of in vivo Administered $^{89}Sr^{++}$ in Rat Liver Subcellular Fractions

A recent study of the subcellular distribution of injected $^{89}Sr^{++}$ in rat liver has shown that, a few min after the injection, mitochondria contained a very large proportion of the total radiostrontium of the cell[1]. Experiments with uncouplers of oxidative phosphorylation have led to the conclusion that energy-linked mechanisms for Sr^{++} uptake are operating in the liver cell. Smaller amounts of $^{89}Sr^{++}$ were associated with the microsomes, and with the fraction that contains the nuclei of the cells. The affinity of the cellular organelles for Sr^{++} is evidently very high, since only negligible amounts of radioactivity were recovered in the cell sap. Preliminary experiments were also carried out on the possible variations in the subcellular distribution of radioactivity as a function of time after the injection of $^{89}Sr^{++}$: shifts in the pattern of distribution were indeed detected, indicating that the subcellular pools of Sr^{++} were not irreversibly sequestered in the organelles, but were to a certain extent interchangeable, and communicating with each other. A sequential transfer of Sr^{++} from one subcellular structure to another could then be a part of the process by which the liver cell assumes, maintains, and discharges the injected $^{89}Sr^{++}$.

A detailed study of the problem has therefore been carried out, with special emphasis on the times immediately following the injection of $^{89}Sr^{++}$. The results obtained have shown that definite changes in its intracellular distribution do indeed occur early after the injection, and that they involve all subcellular fractions studied; it is thus possible to suggest a sequence of events for the fate of Sr^{++} in the liver cell.

Methods. Commercial rats were used, weighing 250–300 g, and fasted 16 h before the sacrifice. $^{89}SrCl_2$ (20 μc/100 g b.w., in 0.5 ml distilled water) was injected i.p. Fractionation and analytical methods have been described

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elsewhere[1]. The 'heavy' microsomes fraction has however been omitted from this presentation. The distribution of $^{89}Sr^{++}$ is expressed on a % basis, referred to the total radioactivity of the homogenate.

Results and discussion. Seconds after injection, $^{89}Sr^{++}$ can be measured in liver. The radioactivity in the homogenate rises in 2–3 min to a plateau that is maintained for at least 60 min (Figure 1). Other experiments have shown that after this time the radioactivity in liver decreases. The distribution of $^{89}Sr^{++}$ among subcellular fractions is variable with time (Figure 2). The main modifications can be described as follows: (a) immediately after injection, the largest part of the radioactive isotope is found in mitochondria. However, the total amount of mitochondrial $^{89}Sr^{++}$ decreases rapidly in the first 3 min, and then more slowly; after 60 min, about 23% of the total $^{89}Sr^{++}$ of the cell is in mitochondria, as compared with a figure of about 57% immediately after the injection. (b) $^{89}Sr^{++}$ in the 'residue' also shows a rapid decrease, that levels off after 2–3 min. The 'residue' is a heterogenous fraction that contains the nuclei of the cells, cell debris, mitochondria, microsomes, and some lysosomes: it is therefore not useful to discuss it as an individual fraction. (c) A minor percentage of the total $^{89}Sr^{++}$ of the cell is found in microsomes immediately after injection; however, in about 3 min this amount doubles, and it increases more slowly thereafter. A plateau is reached in 15–30 min: at this time, almost 20% of the total $^{89}Sr^{++}$ of the cell is recovered in the microsomes. (d) Small amounts of $^{89}Sr^{++}$ are found in the cell sap immediately after injection: however, this amount rises quickly, to account for 15–20% of the total radiostrontium of the cell after 2–3 min. Afterwards, the rise of the $^{89}Sr^{++}$ in the cell sap continues more slowly; after 60 min, almost 40% of the total $^{89}Sr^{++}$ is soluble.

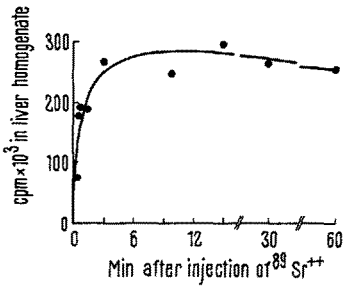

Fig. 1. Radioactivity in rat liver homogenates at various times after injection of $^{89}Sr^{++}$. For technical details see text. Values as cpm in total liver (4.5 g).

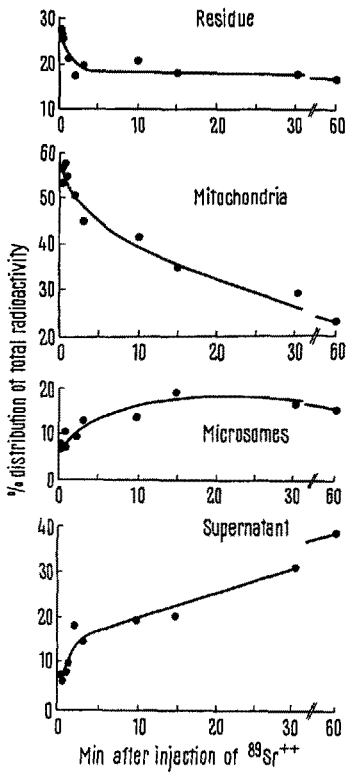

Fig. 2. % distribution of radioactivity among liver subcellular fractions. For technical details see text.

These results indicate that Sr^{++} is not irreversibly sequestered in the organelles of the liver cell, but it is transferred from one organelle to another. Mitochondria seem to be the earliest loci of Sr^{++} uptake, probably because of their high affinity for Sr^{++}, which has been described in in vitro studies[2]. However, they do not maintain Sr^{++} for a long time. Part of the $^{89}Sr^{++}$ ejected by them is taken up by the endoplasmic reticulum, which has only a limited capacity to accumulate Sr^{++}, as shown by the relatively small amount of radioactivity assumed in 15–30 min; in the meantime, mitochondria continue to lose $^{89}Sr^{++}$, part of which therefore remains in the soluble phase. $^{89}Sr^{++}$ in the endoplasmic reticulum is however maintained for a long time: the binding of Sr^{++}, and/or its physical state in the endoplasmic reticulum and in the mitochondria is possibly different. A recent study of the in vivo transport of Ca^{++} in liver has indeed shown that Ca^{++} in the endoplasmic reticulum is in a stable state, while Ca^{++} in mitochondria is in a dynamic steady state. As for the increase of $^{89}Sr^{++}$ in the cell sap at later times, it apparently means that Sr^{++} cannot leave the cell easily.

The deposition of radiostrontium in the animal tissues seems therefore to involve both mitochondria and the endoplasmic reticulum; mitochondria would be responsible for the early uptake of large amounts of radiostrontium from the extracellular fluids, while endoplasmic reticulum would be the locus for a longer-term accumulation of part of the $^{89}Sr^{++}$ set free by mitochondria. Radiation damage could conceivably follow the accumulation of $^{89}Sr^{++}$, due to the presence of DNA and RNA in the organelles[3].

Riassunto. É stata studiata la distribuzione dello $^{89}Sr^{++}$ nelle frazioni subcellulari di fegato di ratto. Nei primi secondi dopo l'iniezione, la maggior parte dello $^{89}Sr^{++}$ si trova nei mitocondri. In alcuni minuti, la radioattività diminuisce nei mitocondri ed aumenta nel reticolo endoplasmico e nella fase solubile. Lo $^{89}Sr^{++}$ viene probabilmente trasferito da una struttura cellulare all'altra; i mitocondri ed il reticolo endoplasmico svolgono ruoli distinti nella deposizione dello Sr^{++} nella cellula epatica.

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