Ringer solution at 37 °C, aerated with 95% $O_2$ and 5% $CO_2$ with a pineal fraction, and 6 male mice hypothalami incubated alone served as a control.

The incubation liquid was used for the incubation with 3 male mice anterior hypophyses for 3 h under the same experimental conditions, to determine the gonadotropin releasing activity. After centrifugation of this second incubation liquid, the supernatant was injected s.c. in 5 injections into 6 immature 21-day-old female Swiss mice of 7–8 g body weight which had been sensitized just before the first injection with 0.25 IU of human chorionic gonadotrophin (HCG). Autopsy was carried out 18 h after the last injection. We compared the average value of the ovary weights and the average value of the uterine weights of the groups. Standard errors of the means were calculated.

The incubated hypothalami, with and without a pineal fraction, were extracted to determine their content of gonadotropin releasing activity by incubating the lyophilized hypothalamic extract with 3 mice anterior hypophyses as described before. For details of the method see MOSZKOWSKA et al.[1].

*Results with fraction F3.* (Original Sephadex G-25 F3 extracted with pyridine acetate, see[2]). We found that the hypothalami of male mice incubated in the presence of the pineal extract secreted less hypophysiotropic hormone than did those hypothalami incubated alone in Krebs-Ringer solution. In addition, the hypothalami incubated in the presence of that Sephadex G-25 fraction F3 had a higher content of hypophysiotropic factor(s) than the control hypothalami. In conclusion, it clearly appears that in the case of the Sephadex G-25 fraction F3, its action on the hypothalamic hypophysiotropic activity is to inhibit the secretion of hypophysiotropic hormones in rat[1] and mice.

*Results with fraction UM-2R.* Hypothalami incubated in the presence of UM-2R showed a highly significant decrease in the secretion of hypophysiotropic hormone compared with the controls. In addition these hypothalami showed a certain decrease in the hypophysiotropic factors content, which contrasts with the situation we found in the case of hypothalami incubated with our original Sephadex G-25 fraction F3 (Figure 1). We can only define the molecular weight of the substances in UM-2R as being greater than 1000. It does not seem possible, therefore, to compare the inhibiting activity in this fraction with that in our original Sephadex G-25 fraction F3.

*Results with the fraction UM-05R.* The hypothalami incubated with UM-05R showed an increased hypophysiotropic activity, since both the incubation medium and the hypothalami ectracts tested following incubation with UM-05R stimulated an increased secretion of pituitary gonadotropin compared with the secretion stimulated by the incubation medium and the hypothalamic extract from control experiments (Figure 2). It seems, therefore, that we are in the presence of a factor which stimulates hypothalamic activity, and which has a molecular weight between 500 and 1000. Thus, we may conclude that the pineal contains active principles, other than melatonin[1], capable of acting via the hypothalamus. It must be noted, however, that BENSON et al.[5] and EBELS and BENSON[4], using a bioassay based on the compensatory ovarian hypertrophy in unilaterally ovariectomized adult mice, have reported that the pineal fraction UM-05R contains an inhibiting activity. With UM-2R they unable to prevent the compensatory hypertrophy. It is therefore evident that with these 2 pineal fractions one must envisage in vivo in addition to in vitro experiments.

*Résumé.* On a étudié sur la souris trois fractions épiphysaires différentes: les fractions Sephadex G-25 F3, UM-2R, UM-05R. La fraction épiphysaire Sephadex G-25 F3 inhibe l'activité hypothalamique hypophysiotrope en empêchant son excrétion. La fraction UM-2R inhibe aussi bien l'excrétion que la synthèse des facteurs hypophysiotropes. La fraction UM-05R se révèle capable de stimuler l'activité hypothalamique hypophysiotrope.

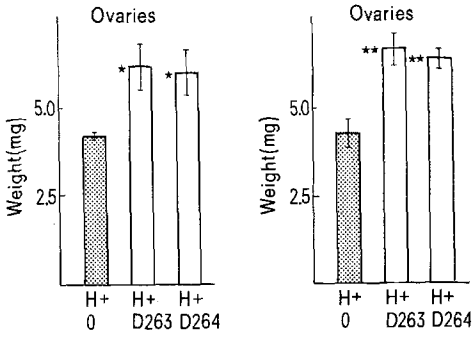


Fig. 2. Weights of the ovaries of mice after injection of the incubation liquid (left) and extract (right) of mice hypothalamus incubated with and without a sheep pineal fraction UM-05R. D 263, D 264 are the codes of two different Sephadex G 25 columns from which UM-05R are prepared. H, hypothalamus. * $p < 5\%$. ** $p < 1\%$.

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## Distribution of Vitamin $D_3$: Evidence of Accumulation in Renal Proximal Tubuli and Thyroid Parafollicular Cells

The conversion of vitamin $D_3$ to the active metabolites, 25-hydroxycholecalciferol (25-HCC) and 1,25-dihydroxycholecalciferol (1,25-DHCC), has been shown to take place in liver and kidney respectively[1,2]. These processes can explain the time lag between the administration of vitamin $D_3$ and its hypercalcaemic effect, proposed to be mediated through an action on intestine and bone[3–5]. In the present investigation we have used whole-body

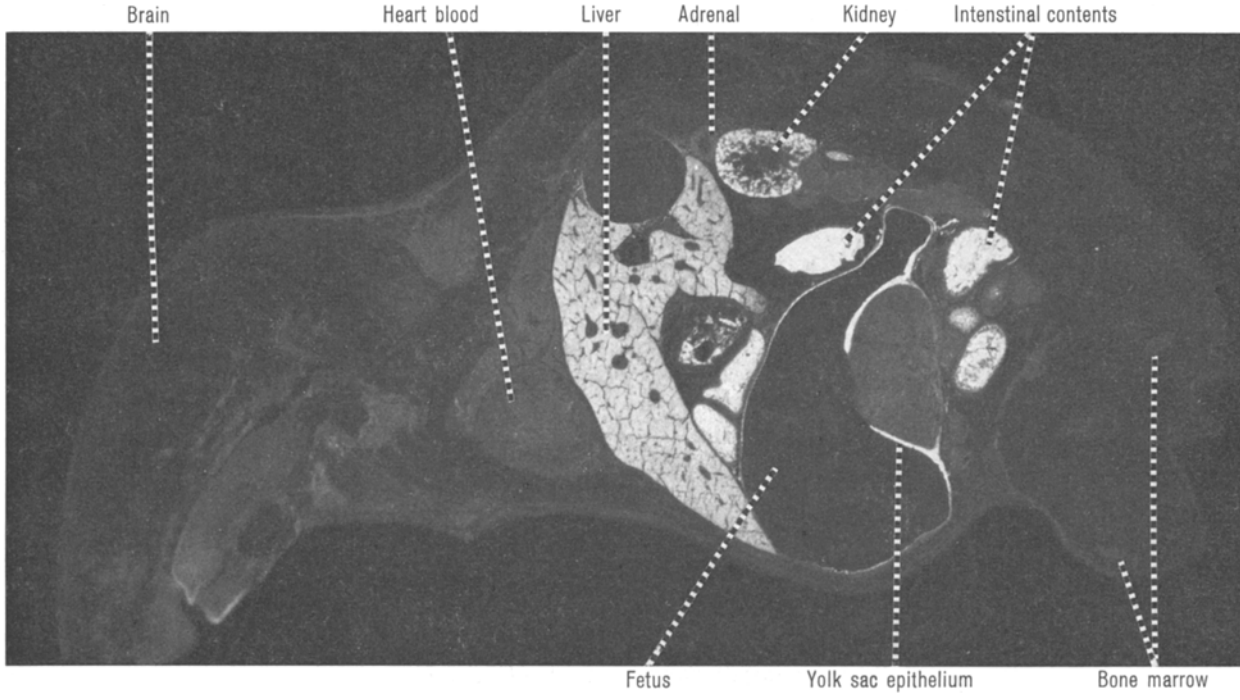


Fig. 1. Autoradiogram of a pregnant mouse 24 h after s.c. injection of vitamin $D_3$-4-$^{14}$C. The highest accumulation of radioactivity (light areas) can be observed in certain parts of the cortex of the kidney, in the yolk sac epithelium and in the intestinal contents. There is also a relatively high radioactivity in the liver. Note the low radioactivity in the fetus.

autoradiography to study the distribution pictures obtained at different time intervals after the injection of $^{14}$C-labelled vitamin $D_3$ to mice. Our aim has been to determine whether the distribution patterns can be correlated to presumed sites of metabolism of vitamin $D_3$ and possible sites of physiological action.

Two male and 2 pregnant female C57BL mice were injected s.c. with vitamin $D_3$-4-$^{14}$C (The Radiochemical Centre Amersham, spec. act. 83,8 μCi/mg; dose/animal 12,5 μg) dissolved in 0.1 ml dimethyl sulfoxide. The animals were killed at 1 h (male) 8 h (male), 24 h (female), and 96 h (female) after injection. Autoradiography of

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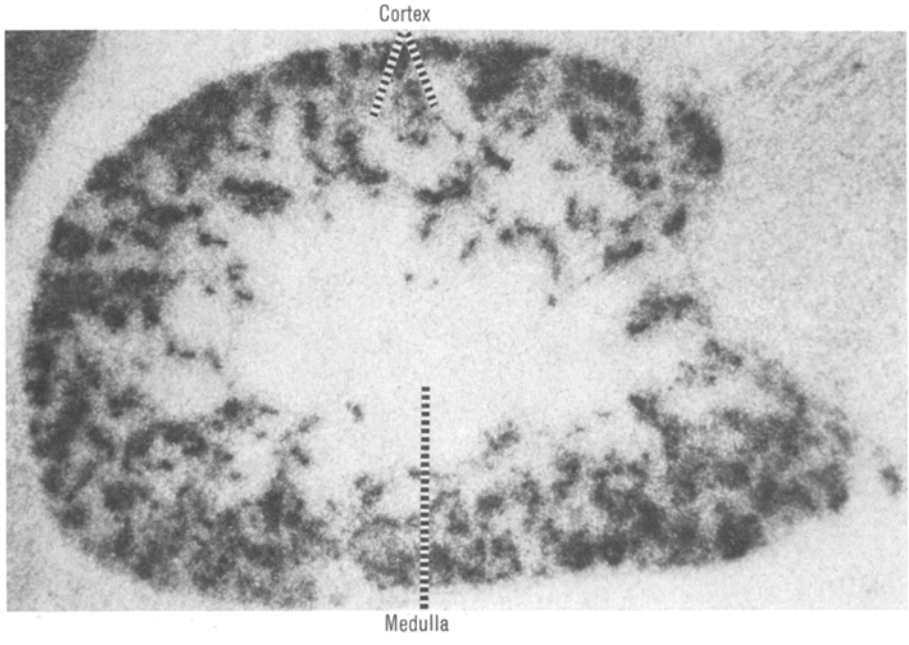


Fig. 2. Detail of whole-body autoradiogram of a mouse 24 h after s.c.injection of vitamin $D_3$-4-$^{14}$C, showing the accumulation of radioactivity in the kidney. There is a high radioactivity in certain areas of the labyrinthic part of the cortex, most probably the convoluted portion of the proximal tubuli.

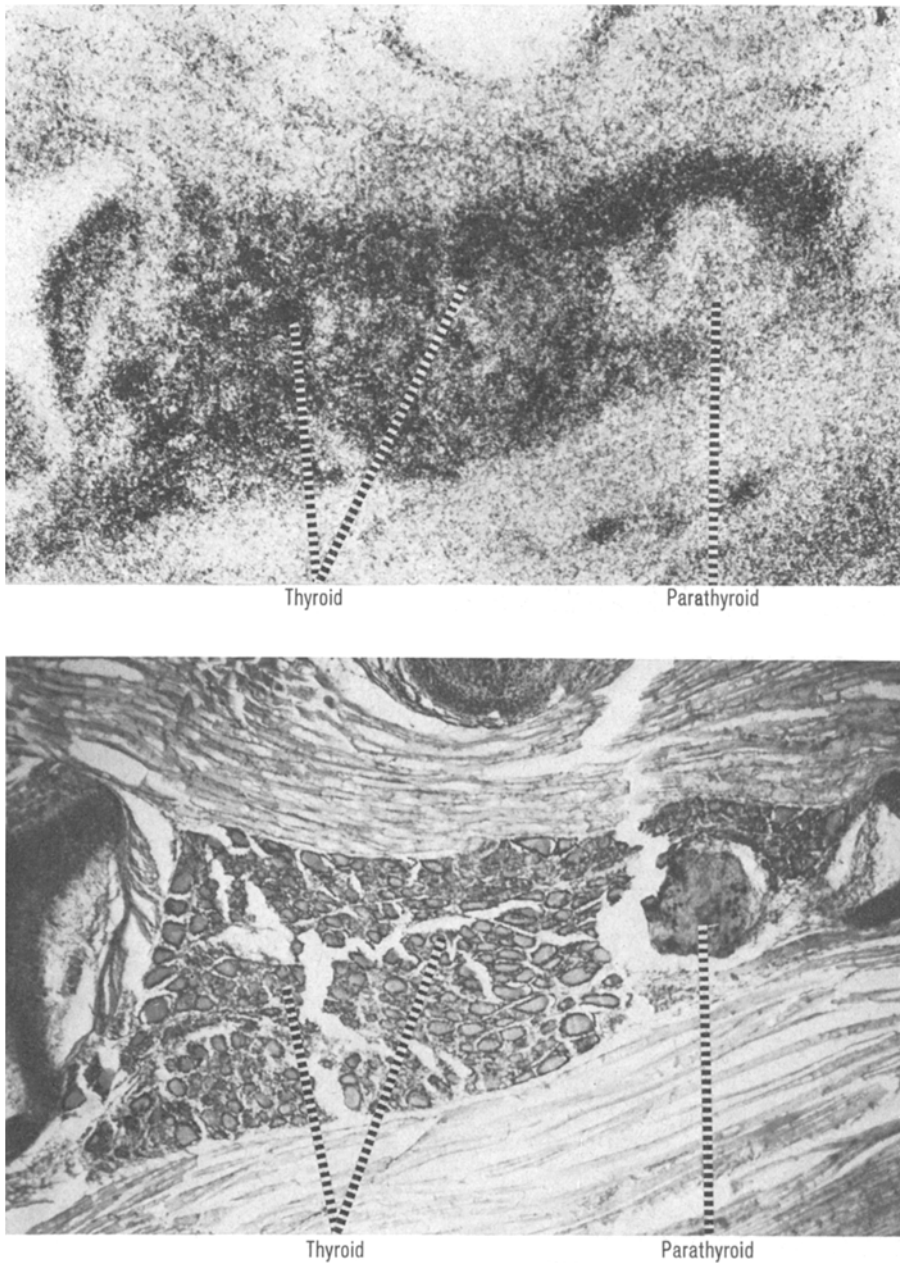


Fig. 3. a) Detail of a whole-body autoradiogram of a mouse 8 h after s.c. injection of vitamin $D_3$-4-$^{14}$C. b) The corresponding section stained with haematoxylin-eosin. The uptake of radioactivity in the thyroid is highest at certain localities which do not entirely coincide with follicles, and it is thus possible that the activity represents an accumulation in the calcitonin-producing parafollicular cells. The radioactivity in the parathyroid is low.

whole-body sagittal freeze-dried sections of the mice[6] was performed at −15 °C to avoid diffusion of fat-soluble material[7]. The order of isotope concentration in the organs was determined by densitometric comparison with a standard isotopic staircase[8].

The autoradiograms showed that at short survival times (1 and 8 h) there was a high accumulation of radioactivity in the liver, while the uptake in the kidneys was relatively low and evenly distributed. At longer survival times, in contrast, there was a high and well localized uptake in the kidneys while the concentration in the liver was considerably lower (Figur 1, Table). Within the kidneys the radioactivity was concentrated to certain areas of the labyrinthic part of the cortex, most probably the convoluted portion of the proximal tubuli (Figure 2). The radioactivity was low in the pelvis of the kidney and in the urinary bladder. The delayed uptake of the radioactivity in the kidneys indicated that a metabolite, and not vitamin $D_3$ itself, was responsible for the accumulation observed here. It is possible that this metabolite is 25-HCC, which is known to be converted to 1, 25-DHCC in the kidneys[2]. 1, 25-DHCC, which has been considered to be a hormone[5], might also be stored at its place of formation.

In bone there was a markedly low uptake during all the observed time intervals, both in permanent bone and in growth zones. On the other hand, there was an accumulation in connective tissue associated with bone, and also elsewhere, and in bone marrow. It is possible that in non-rachitic animals the active metabolites are present in low concentrations in bone cells, which are considered to be

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target cells for the vitamin. If the uptake of vitamin D in bone is to be measured by quantitative determination of extracts from homogenates, the relatively high uptake in bone marrow and surrounding connective tissue might be interpreted erroneously as an accumulation in bone. The accumulation of radioactivity in connective tissue may be of significance with regard to the reported stimulatory effect of vitamin D on bone collagen synthesis[9].

There was only moderate accumulation of radioactivity in the intestinal mucosa during the whole observation period. Because of the high intestinal radioactivity content, and because of a probable absorption of radioactivity from the lumen, the significance of these results is difficult to interpret.

An interesting finding was the relatively high accumulation of radioactivity in certain endocrine organs such as the thyroid, hypophysis, and adrenal cortex. As can be seen in Figure 3, the uptake in the thyroid is highest at certain localities, which do not entirely coincide with follicles, and it is thus possible that the activity represents an accumulation in the calcitonin-producing parafollicular cells. The activity in the parathyroid, however, is low (Figure 3). Calcitonin has been shown to enhance the production of 1,25-DHCC in the kidneys of rats[10]. Vitamin D causes a degranulation of the parafollicular cells in cows also in the absence of significant hypercalcaemia[11]. A direct effect of vitamin D on the parafollicular cells might be considered as a possible link in the complicated regulation of the calcium-homeostasis.

In the fetus, the concentration of radioactivity was very low 24 h after injection. However, after 4 days the concentration was as high as in the mother, and the distribution pattern was also similar to that seen in the mother at this long time after injection. Thus, there was a localized uptake of radioactivity in certain areas of the kidney. The uptakes in thyroid, adrenal cortex and hypophysis seemed to be relatively a little higher than in the mother. The delayed uptake in the fetus may indicate that a metabolite, and not vitamin $D_3$ itself, is transferred to the fetus. The accumulation in the fetal kidney may also suggest that the fetus has the ability to convert 25-HCC to 1,25-DHCC. It has been claimed that the fetus cannot metabolize vitamin $D_3$ and that 25-HCC rather than vitamin $D_3$ passes to the fetus[12]. There was also an accumulation of radioactivity in the yolk sac epithelium, as has been found for a number of other substances[13]. Its importance is, however, not well understood.

Semiquantitative evaluation of whole-body autoradiograms after s.c. injection of vitamin $D_3$-4-$^{14}$C

| | Time (h) | | | |
|---|---|---|---|---|
| | 1 | 8 | 24 | 96 |
| Kidney (parts of cortex) | 16 | 128 | 1024 | 1024 |
| Liver | 128 | 256 | 256 | 128 |
| Mucosa of small intestine | 128 | 128 | 128 | 128 |
| Thyroid | 8 | 128 | 128 | 64 |
| Hypophysis | 8 | 128 | 128 | 64 |
| Adrenal cortex | 8 | 128 | 128 | 64 |
| Brown fat | 8 | 128 | 128 | 128 |
| Bone marrow | 4 | 128 | 128 | 32 |
| Connective tissue | 4 | 16 | 128 | 128 |
| Blood | 4 | 16 | 64 | 32 |
| Intestinal contents | 512 | 512 | 1024 | 512 |

The radioactivity in different organs was compared with autoradiograms of simultaneously exposed $^{14}$C-isotope-staircases[8]. These consisted of 10 steps of increasing isotope concentration in the geometric series $2^1$ (2), $2^2$ (4), $2^3$ (8) ... $2^{10}$ (1024). The radioactivity for the different localities is expressed as the relative isotope concentration of the staircase step with which it matched. The radioactivity in bone never exceeded the lowest step of the staircase, and has therefore not been included in the table.

*Zusammenfassung.* Mit Hilfe der Ganzkörper-Autoradiographietechnik konnte nach Zufuhr von Vitamin $D_3$-4-$^{14}$C an Mäusen der Nachweis einer radioaktiven Ansammlung in gewissen Gebieten (Labyrinth) der Nierenrinde sowie der Thyroidea erbracht werden, die möglicherweise mit parafollikulären Zellen identisch ist.

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## Asymmetrical Responses to Directional Selection for Radiation Resistance and Sensitivity in *Drosophila*

Genetic variation for resistance and sensitivity to extremely high doses of $^{60}$Co $\gamma$-radiation has been reported in *D. melanogaster*[1-3]; as assessed by scoring percentage mortality after short periods of time. In this article we report on an attempt at exploiting such genetic variation by carrying out directional selection. Since the high doses of radiation used lead to immediate sterilization and early death, it was necessary to employ a method of sib-selection[4] where selection is based only on the values of relatives. A response to selection in such circumstances implies that the hereditary variability must already be present in the population, as the selected flies are never themselves exposed to the selective agent.

The experiments were started from strains set up from single inseminated females collected from a population polymorphic for radioresistance at Leslie Manor (LM) near Camperdown, Victoria in 1965[2]. Initially, 3 resistant LM strains were crossed together and 3 sensitive LM strains

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