

A MICRO SUBLIMATION STEP IN THE UV SPECTROPHOTOMETRIC DETERMINATION OF DIAZINON

THE microdetermination of diazinon¹⁻³ through 2-isopropyl-6-methyl-4-pyrimidinol is tedious; further the presence of animal and vegetable co-extractives complicate the analysis. In this communication a micro sublimation step is described for the purification of the pyrimidinol before UV determination.

2-isopropyl-6-methyl-4-pyrimidinol sublimes to white crystalline needles. The sublimation of the pyrimidinol is carried out by heating at $115 \pm 1^\circ \text{C}$ for 10 minutes and is thus purified from co-extractives which in effect do not volatalise at this temperature.

The sublimation assembly (Fig. 1) consists of a metallic hot air-bath top covered with an asbestos sheet.

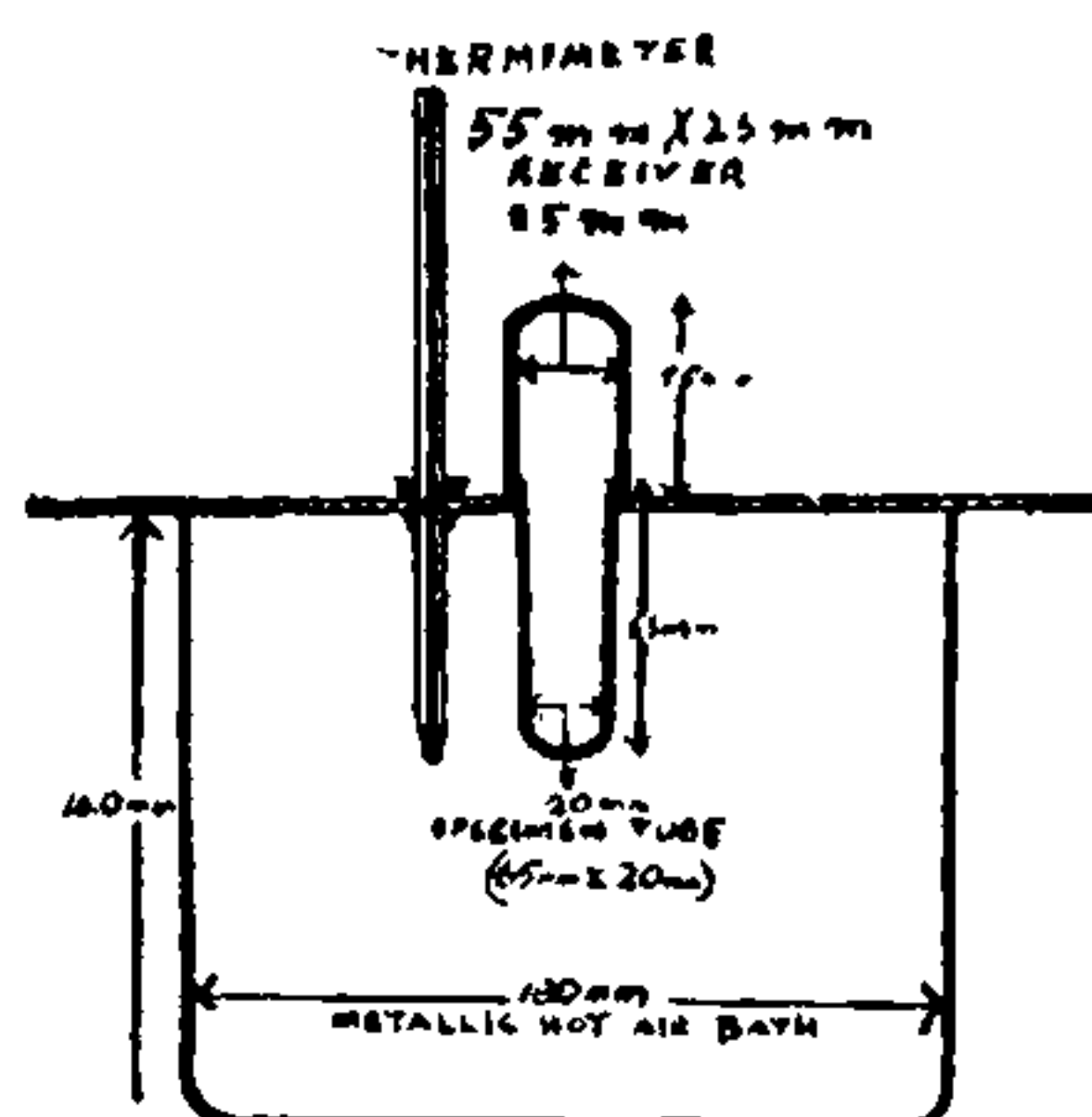


FIG. 1. Diagram showing the sublimation assembly.

An unspouted specimen tube fits the central hole of the asbestos cover, through another hole passes a thermometer. The receiver is another unspouted specimen tube of a slightly larger diameter. The bath may be heated either electrically or by flame at the rate of about 10°C per minute.

The contaminated pyrimidinol requiring purification is taken in the specimen tube, and the sublimation carried out at $115 \pm 10^\circ \text{C}$ for 10 minutes and then the apparatus is allowed to cool to the ambient temperature. The sublimate is dissolved in 95% V/V ethanol and absorbance measures at 272 nm. The quantity of diazinon is then determined from a calibration curve prepared from purified pyrimidinol. One mg of pyrimidinol is equivalent to 1.657 mg of diazinon. The calibration curve obeys Beer's Law over the range of 0-80 ppm of pyrimidinol and the molar extinction coefficient is 4864.

For recovery studies known quantities of the pyrimidinol were spiked with about 500 fold excess

of tissue extractive and the recovery by sublimation determined. Recovery of the pyrimidinol by the proposed method was satisfactory and reproducible and was found to be 99.25% with a standard deviation of $\pm 0.75\%$ upto $100 \mu\text{g}$ level. Sublimation of quantities greater than $100 \mu\text{g}$ results in substantial loss of the pyrimidinol and is therefore not recommended. It was found that a slight variation in the sublimation assembly did not significantly affect the recovery.

This purification step by micro sublimation should find an extensive application in the determination of diazinon in matrices such as animal tissue, vegetable matter, etc., giving copious co-extractives.

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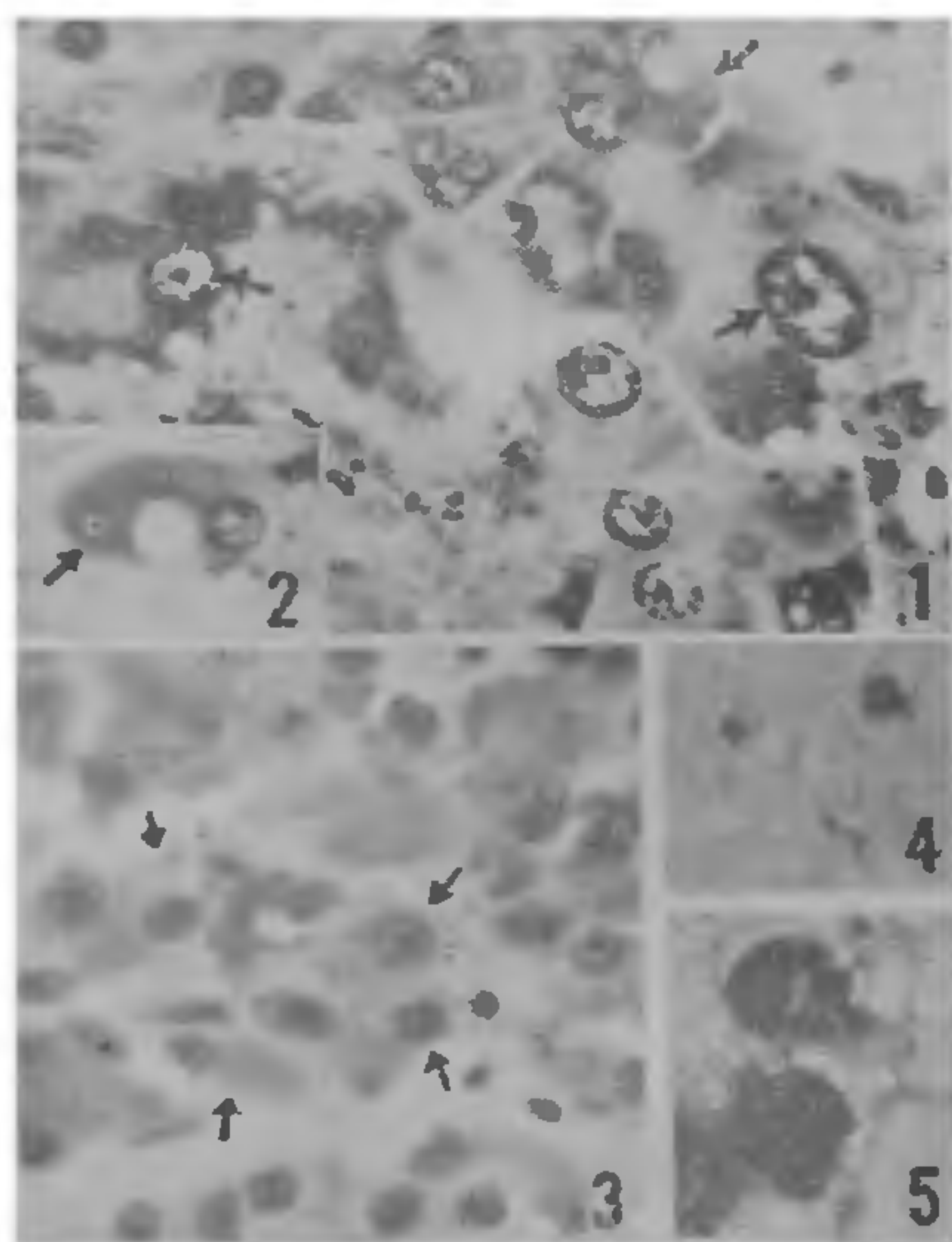
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DISTRIBUTIONAL PATTERN OF THYROTROPHS IN THE PITUITARY OF HETEROPNEUSTES FOSSILIS (BLOCH.) BASED ON RADIOTHYROIDECTOMY

ALTHOUGH the pituitary of teleosts has been extensively studied^{6,1,7,8,4} lack of agreement about the function of the various pituitary cell types is obvious. It is largely due to the fact that experimental allocation of function to the cells has been attempted systematically only in a few species¹. In the teleosts the distributional pattern of the thyrotrophs greatly vary among different species. In some they are situated in the proximal pars distalis², whereas in others they are in the rostral pars distalis³ or even between the two zones⁵.

In the present investigation 10 *H. fossilis* were given $250 \mu\text{Ci}$ of I-131 in two instalments at an interval of six months and sacrificed after the completion of a full year. The pituitary was fixed in Bouin's fluid containing 5 gm of Mercuric Chloride; sections were cut at 5μ thick and stained in combination of PAS and lead haematoxylin (PbH), aldehyde fuchsin (AF) with Halmi's fastgreen-Chromotrope 2R-Orange G and Mallory's triple stain. Thyroid was fixed in Bouin's fluid, stained in PAS and Ehrlich haematoxylin and autoradiographs were prepared using NTB 3 Kodak emulsion.

In only one fish remnants of the thyroid was present in the form of 3 or 4 scattered microfollicle which gave positive autoradiograph (Fig. 4). In the others as no viable thyroid tissue was present autoradiographs were negative. In the partial thyroidectomised fishes as the thyroid is in the form of microfollicle autoradiograph is very small when compared to the controls (Fig. 5). Both in partial and total radiothyroidectomised fishes the pituitary thyrotrophs exhibited extensive compensatory hypertrophy. They measure 14μ in diameter in comparison to control which measure only 7μ (Figs. 1 and 3). The activated nuclei is round with prominent nucleoli. Several of them have large vacuoles in their cytoplasm (Figs. 1, 2).



FIGS. 1-5. Figs. 1, 2. Arrows show hypertrophied thyrotrophs in the radiothyroidectomised fish, $\times 800$. Fig. 3. Arrows show thyrotrophs in a normal fish, $\times 800$. Fig. 4. Autoradiograph of the thyroid in a partially thyroidectomised fish, $\times 80$. Fig. 5. Autoradiograph of three thyroid follicles in a normal fish $\times 80$.

These hypertrophied cells sometimes lack a distinct cell boundary and groups of them look like a syncytium. In the control specimens it is difficult to differentiate the thyrotrophs from the gonadotrophs as both were stainable with PAS, AF, aniline blue and fastgreen. In this species the thyrotrophs are localised in the middle of the proximal pars distalis flanked by gonadotrophs on either side. The acidophilic somatotrophs are distributed throughout the proximal pars distalis.

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EFFECT OF A COPPER INTRA-UTERINE CONTRACEPTIVE DEVICE ON SUB-CELLULAR DISTRIBUTION AND CONCENTRATION OF COPPER IN THE RAT UTERUS

COPPER containing intrauterine contraceptive device (Cu-IUD) has been reported to have better contraceptive efficacy and minimum side-effects than the polyethylene IUDs. The primary mode of action of copper has been attributed to its effect on endometrium and alteration of uterine fluid milieu both in animals and woman¹. The catalase enzyme activity in the endometrium of rat is reported to be stimulated by Cu-IUD². The presence of copper in cervical mucus inhibits sperm penetration³. There is non-uniform dissolution of copper ions from wire and even flakes of copper are shed from the wire⁴ but the overall release rate of copper from device is 10.3 mg/year ⁵ and its concentration is significantly higher in the late secretory phase in woman⁶. It is pertinent to mention that copper content of liver, lungs, etc., remains unaltered after Cu-IUD signifying its local effect⁴. The subcellular localisation of copper in the uterus is still unknown.

Accordingly, the present investigation deals with the subcellular distribution of copper in rat uterus fitted with a copper IUD.