PN 1211

Detection of steroids on chromatoplates using a non-destructive method

The excellent resolution and rapid development times of thin-layer chromatography make this technique a very useful tool in the steroid field. As many steroids have characteristic absorption bands in the ultraviolet *e.g.* cortisone, progesterone, cholestenone etc., advantage may be taken of this fact to develop a method whereby these substances can be detected, and recovered quantitatively from a thin-layer plate without the use of a destructive spraying reagent. This is of importance in steroid studies *in vitro* where the material has to be quantitatively eluted, chemically determined and also assayed for radioactivity.

Thin-layer chromatography was carried out in the usual way except that quartz plates were used instead of glass plates. The plates were viewed with a mercury lamp (maximum emission 254 mμ) and ultraviolet-absorbing substances appeared as dark spots. However, the silicic acid layer itself absorbed (or scattered) the incident light and so the contrast between spots and background was not very great. This meant that the method had a low order of sensitivity.

SEASE[1] incorporated an inorganic phosphor into silicic acid and used the mixture in a quartz column. By viewing the column under ultraviolet light he observed the presence of ultraviolet-absorbing bands. KIRCHNER[2] adapted this method to thin-layer chromatography of terpenes using glass plates for the chromatography.

This method was modified to permit its use in the detection of steroids as follows. Various inorganic phosphors which fluoresce green under the mercury ultraviolet lamp were mixed with Silica Gel G (Merck) and used for thin-layer chromatography of steroids on glass plates. The silicic acid layer was exposed to an ultraviolet source and the chromatoplate examined from the "glass side". Absorbent substances having maximum absorption between 240 and 280 mμ show up as dark spots which can be marked on the glass side, eluted and either estimated spectrophotometrically and/or assayed for radioactivity. Since the wavelength of the emission from the phosphor varies with the wavelength of the ultraviolet light used to illuminate the plates the background colour changes with some phosphors from bluish green at 200 mμ to green at 240 mμ and to red at 300 mμ.

This method is particularly applicable to the study of intermediary metabolism of adrenal steroids and oestrogens and also to the study of the breakdown of cholesterol to bile acids where several of the expected intermediates are ultraviolet absorbing. For example, using this thin-layer technique coupled to a preliminary column method it can be shown that incubation of 7α-hydroxycholesterol with certain cell fractions of rat liver gives rise to cholest-4-en-3-one-7α-ol (*cf.* YAMASAKI[3]; DANIELSSON[4]) with maximum absorption at 242 mμ, while incubation of cholest-4-en-3-one-7α-ol produces a more polar ultraviolet-absorbing substance (maximum absorption at 240 mμ) which is possibly choiest-4-en-3-one-7α-26(?)-diol. Using this method, incubations can be carried out with very small amounts, in some cases less than 25 μg of substrate either unlabelled or labelled with tritium and the products of the reaction detected and quantitatively estimated. These substrates and products cannot be separated as quickly or in such small amounts using conventional chemical methods.

The method can be made more specific by varying the wavelength of the ultraviolet light used to illuminate the plate. High-intensity ultraviolet sources and gratings

are required for this purpose because the energy of the monochromatic light emerging must be considerable to illuminate effectively even small plates.

Using a hydrogen lamp, substances which maximally absorption at 207 mμ such as cholesterol and 7α-hydroxycholesterol, can easily be detected. Thus by using a series of interference filters in the range 200–300 mμ with a hydrogen source, this method not only shows the position of a substance on the plate but indicates its ultraviolet-absorbing properties and thus gives information on its possible molecular structure.

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PN 1210

The effect of thyroid hormones on oxygen consumption of isolated horse leucocytes

As part of a continuing study of the effects of thyroid hormones and their attendant molecular alterations at various levels of biological organization, we have studied the oxygen consumption of isolated horse-blood leucocytes. Previous studies have indicated: (1) a correlation of O_2 consumption of human leucocytes with the thyroid status of the donor[1,2] (2) increased leucocyte O_2 consumption after administration *in vivo* of thyroid hormone[3] and (3) response *in vitro* to triiodothyroacetic acid of myeloid leukemic leucocytes[4].

This report deals with a prompt increased O_2 consumption of horse leucocytes by addition *in vitro* of L-triiodothyroacetic acid and a lesser and delayed increase with L-thyroxine and L-triiodothyronine.

Leucocytes were harvested from 4 l of horse blood by the methods of MAUPIN[5], avoiding the addition of any substance other than crystalline heparin, yielding $0.8 \cdot 10^{10}$–$3.2 \cdot 10^{10}$ leucocytes containing less than 1 erythrocyte per 15 leucocytes. The O_2 consumption of $10 \cdot 10^7$–$20 \cdot 10^7$ leucocytes was determined, in triplicate, by standard manometric techniques immediately after preparation and after storage at 0° for 4, 24, and 48 h (expressed as μmoles O_2 consumed per h per 10^{10} leucocytes). Thyroid hormones, solubilized in 2–3 drops of NaOH, were added just before the run or from the side arm after 15-min equilibration.

In Krebs–Ringer phosphate media[6], O_2 consumption ranged from 33.2 to 91.5 μmoles O_2/h/10^{10} leucocytes, averaging 55.0 ± 15.8. Suggestive increases were observed with thyroid hormone addition ($2 \cdot 10^{-6}$–$5 \cdot 10^{-6}$ M) but were small and variable (9 to 22 % with triiodothyroacetic acid, 0–10 % with triiodothyronine or thyroxine).

In subsequent runs using a buffer medium devised by DICKENS AND SALMONY[7] (see Table I) O_2 consumption was strikingly higher (from 98.6–225 μmoles O_2/h/10^{10}