RADIOCHEMISTRY

Autoradiography of Tritium-labelled Compounds on Paper Chromatograms

THE standard method of rendering visible radioactive compounds on paper chromatograms is to place the paper against a fast X-ray film for the requisite exposure period. The energies of β-particles emitted by tritium are so low (E_{max} = 18 keV.) that the paper itself and the inevitable air gap between paper and film absorb most, if not all, of the radiation, reducing the sensitivity of the technique below useful limits. Wilson[1] has described the preparation of scintillation autographs, in which the chromatographic strip, in close contact with a fast film, is immersed in a tank of scintillation fluid; but his method cannot be applied to the study of compounds, such as steroid hormones, which are soluble in toluene, the primary solvent of most scintillating fluids.

It seemed that a possible technique would be to coat the paper chromatogram directly with melted photographic emulsion, which would penetrate into the paper, and come into very close contact with the tritiated compound. Tritiated progesterone (Radiochemical Centre, Amersham, Code No. *TRA*-11), was chosen for preliminary tests. The chromatograms were very kindly prepared by Dr. G. H. Thomas: various amounts of labelled progesterone of known activity were spotted on Whatman No. 1 paper, and chromatographed in the Bush *A* solvent system[2]. After drying, the strips were taken to the dark-room and passed through a trough of melted photographic emulsion at 50° C. To facilitate subsequent handling, the strips were next fixed to hangers of the type used for holding X-ray films. They were hung up at room temperature for 2 hr. to allow excess emulsion to drip off, and the remaining emulsion to dry; then placed in a light-proof box with about 20 gm. of dried silica gel, and left in a cold room at 5° C. for periods ranging from 1 to 4 weeks. They were finally processed in tanks of standard X-ray developer and fixer, washed for 2 hr. in running water, and dried.

Ilford *K*2 emulsion is very satisfactory for this work. It is a nuclear research emulsion of reduced sensitivity, suitable for recording low-energy electrons, and having a low background level of developed silver grains in areas unaffected by radioactivity. Coating the strips is simplified if the emulsion is diluted in the trough by an equal volume of distilled water: this does not appear to affect the properties of the emulsion, and gives a thin uniform spread over the paper. Strips prepared with diluted emulsion in this way need 5–6 min. in developer, and 10–15 min. in fixer. Undiluted emulsion gives a thicker and sometimes uneven coverage, and needs longer development and fixation. In neither case are the spots noticeably smeared by immersion in the emulsion.

Many levels of activity have now been tried, and those activities from 0·1 μc. of tritiated progesterone up to 5 μc. have consistently produced black spots of developed silver, readily visible, and increasing in density with increasing activity. With undiluted emulsion and four weeks exposure, 0·07 μc. has been visualized and 0·01 μc. has on one occasion given a recognizable spot. Control chromatograms with unlabelled progesterone did not produce any blackening of the emulsion.

At the specific activity being used, the spots of 0·1 μc. represent about 0·15 μgm. of progesterone. The technique is obviously very sensitive, and as more tritiated compounds become available at high specific activities, it would seem to have many possible applications.

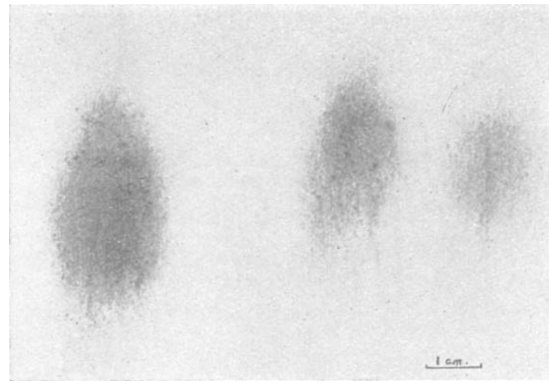

Fig. 1. Portion of chromatogram covered with diluted *K*2 emulsion showing from left to right, 3·3 μc., 0·66 μc., and 0·33 μc., respectively of tritiated progesterone, after 2 weeks exposure.

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[1] Wilson, A. T., *Nature*, **182**, 524 (1958).
[2] Bush, I. E., *Biochem. J.*, **50**, 370 (1952).

PHYSIOLOGY and BIOCHEMISTRY

Effect of Diet on the Blood Sugar and Liver Glycogen Level of Normal and Adrenalectomized Mice

WHILE investigating the effects of adrenal steroids on the blood sugar and liver glycogen levels of mice we were uncertain whether the previous diet of the mice affected these levels. Different types of food were therefore fed to groups of normal and adrenalectomized mice after which the blood sugar and liver glycogen levels were estimated.

Male white mice of the Swiss strain (15–20 gm.) obtained from the Agricultural Research Council were adrenalectomized under ether anæsthesia. On the morning of the second post-operative day the adrenalectomized animals together with groups of normal (non-adrenalectomized) mice were fasted for 24 hr. to deplete the liver of glycogen and lower the blood sugar to a resting level. During this time they had free access to drink; water for the normal mice and saline for those that had been adrenalectomized. Groups of ten mice were used. At the end of the fasting period one group of normal and one group of adrenalectomized mice were killed and used as controls and the remainder given their special diet *ad lib.*

Three types of food were used: (1) a cube diet (M.R.C.41 *b*); (2) pure glucose and (3) pure protein (casein). The groups of mice were killed at logarithmic time intervals and the biochemical estimations made. The blood sugar levels were measured by the method of Hagedorn and Jensen and the liver glycogen by that of Block and D'Arcy[1]. The effect of one of these diets (cubes) is shown in Fig. 1. Both the blood sugar and the liver glycogen content rise rapidly reaching a peak value after 8–16 hr. They then fall to a lower level. The result of the glucose diet is similar except that the peak blood sugar levels are reached in only one hour. Both the glycogen and blood sugar peaks are almost identical quantitatively with those obtained with a cube diet. The effects produced by the pure protein