CHROM. 3325

A rapid method for the estimation of seromucoid synthesis

It is well known that in disease states the serum glycoprotein concentration shows a marked rise[1], probably due to the stimulation of hepatic synthesis by an unknown mechanism[2]. The estimation of the rate of synthesis is more meaningful in the experimental investigation of this problem than are changes in the serum level, but the usual isotopic methods are rather laborious for use in the large numbers of animals required. A rapid method for screening tissue extracts and other substances for their ability to affect the synthesis of seromucoids in mice has therefore been devised, based on the observation that the α-globulin fraction incorporates sulphate[3].

The mice are injected with 50 μC of ^{35}S as sulphate and bled 24 h later. This time interval allows maximum incorporation with little recirculation. A 50 μl sample of serum is placed on Whatman No. 3 M paper strips 5 cm wide, and electrophoresis is carried out in the usual way in barbitone buffer pH 8.6, for 18 h at 2 mA per strip. The papers are dried and scanned in a Packard gas flow counter with 4π configuration, using an 0.5 cm slit. A typical scan is shown in Fig. 1. As stated below, some activity seems to be associated with the transferrins, and therefore only the area of the peak from the edge of the β zone up to the end of the trace is measured and the activity calculated from this. Two standards of 0.1 μC are applied to paper and their mean activity similarly calculated. The results are expressed as the percentage of the original dose per 10 ml of serum.

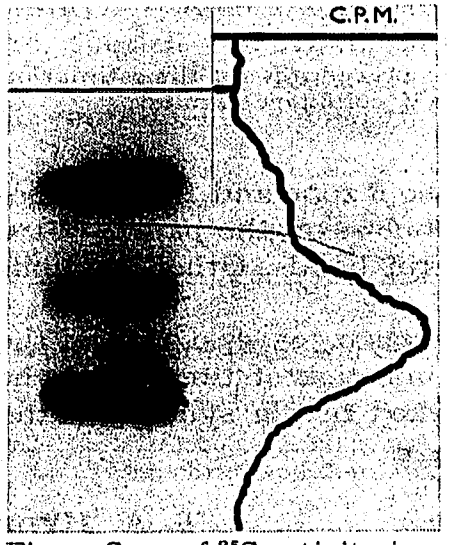

Fig. 1. Scan of ^{35}S activity in mouse serum proteins after paper electrophoresis.

That inorganic ^{35}S is cleared from the strip during electrophoresis was shown by adding the labelled sulphate to a normal serum. After electrophoresis no detectable activity was present on the strip, whereas with a labelled serum, approximately 50 % of the total serum activity remained on the strip. The reproducibility of the method was determined by repeated electrophoresis of a labelled sample; the mean activity of 8 separations was 246 $\pm$ 1 c.p.m. (S.D. $\pm$ 1.0 % of mean). The electrophoresis method was compared with the more lengthy extraction procedure, in which the serum is

dialysed for 72 h, the seromucoid precipitated and digested, and the activity subsequently counted by a scintillation method using a dioxan phosphor[4]. The comparison is shown in Fig. 2, together with the regression line and 95 % confidence limits; the correlation is statistically highly significant ($P < 0.001$).

In an attempt to define more precisely which fractions were incorporating the isotope, labelled samples were run in a standard vertical starch-gel system[5]. Gasflow scanning of a gel strip showed the major part of the activity to be present in the fast α_2 and post-albumin zones. Two-dimensional paper and starch-gel electrophoresis was also carried out, with subsequent elution and scintillation counting of individual areas. Approximately 25% of the total serum activity was present in the post albumin zone,

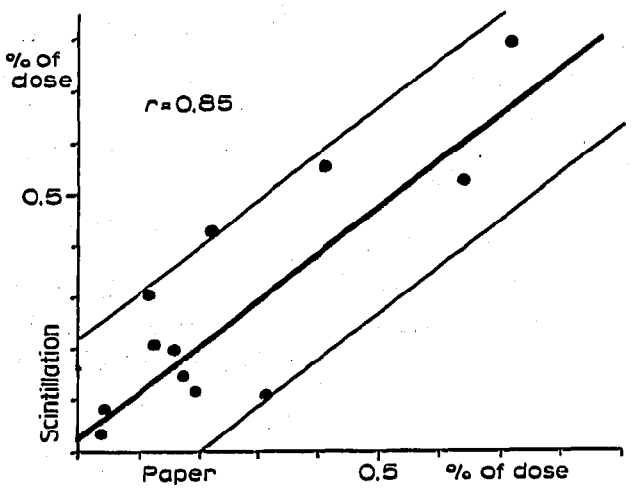

Fig. 2. Comparison of paper electrophoresis and liquid scintillation procedures for the estimation of incorporated ^{35}S in mouse serum. (Fitted regression line with 95% confidence limits).

and 12 % in the fast α_2 zone. Some 10% was present in the β zone and from its position could have been due to trailing of the faster α globulins on the paper, but a further 15 % was definitely associated with the transferrin bands. Other fractions showed negligible activity.

By this method the mean normal value for 17 mice was 0.595 $\pm$ 0.05 % of the original dose per 10 ml of serum, and in 8 mice given an injection of turpentine the value was 1.029 $\pm$ 0.14 %, indicating the expected marked rise in synthesis rate, ($P < 0.005$).

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Received November 17th, 1967