Interaction of normal human serum with [^{131}I]insulin

The addition of [^{131}I]insulin to diabetic serum *in vitro* is one of several approaches that have been used in investigating the fate of insulin in the diabetic subject. Binding of the labelled insulin to γ- or inter $\beta\gamma$-globulins is found in diabetic serum but not in normal serum[1–5].

Binding to other normal serum protein fractions has been reported, and attributed to radiation damage of the insulin[1,7]. Radioactivity in the α_2-globulin region, increasing with time on incubation at 37° (ref. 1), was later stated to be due to degradation of the [^{131}I]insulin by serum[6]. However, no evidence for this was presented and the effect has attracted little further attention, though α_2-binding has been found by MITCHELL[5].

This effect had also been observed during earlier work in these laboratories on diabetic and normal patients[5] and in view of its possible relevance to the transport and fate of insulin in the normal human we have investigated it in more detail.

Samples of normal human serum (100 μl) were mixed with [^{131}I]insulin (4 μg; 1 μC; 10 μl), incubated under various conditions of time and temperature, subjected to paper electrophoresis (Whatman 3MM; sample applied near cathode end; Veronal–NaCl buffer, pH 8.6, I 0.1; 16 h at 5 V/cm), fixed and stained (3% sulphosalicylic acid + 0.02% Fast Green), dried and autoradiographs prepared with a standardised exposure[9]. Radioactive zones on the papers were cut out, disintegrated in 5 ml of 2 N NaOH in counting vials and the activities measured by scintillation counting.

With many specimens of normal human serum no radioactivity appeared in any protein fraction when electrophoresis was carried out soon after mixing the serum and labelled insulin, all the insulin remaining near the origin. This was found with insulin labeled with ^{131}I by four methods[2,10–12] and indicates that there is no radiation damage to the insulin such as that reported by workers[1,13,14] using higher specific activities than ours. Sometimes a small amount of radioactivity, less than 1% of the total in the sample, was found in the α_2-globulin region.

Tracer amounts of the labelled insulin run without serum showed one radioactive zone only, at the origin.

On incubation of the serum–insulin mixture at 35°, radioactivity appeared in the α_2-globulin region, and in that region only. (This radioactivity is not necessarily associated with the α_2-globulin but will be referred to as "α_2-activity" for convenience.) It has been found consistently with all of the many normal human sera examined over the course of several years and contrasts with the findings of BERSON AND YALOW[6] that "many serums are relatively free of such [insulin-degrading] action". The effects of time and temperature of incubation on the appearance of α_2-activity are shown in Fig. 1. Little is apparent after incubation at 4° or 20° for 3 days but at 35° it is detectable after a few hours and after 3 days accounts for more than 40% of the radioactivity in the sample. Little difference was found between electrophoretic runs at 4° and at 20°.

The general picture is the same whether the labelled insulin is used immediately after preparation or after storage until the specific activity is becoming unusably low. The effect is thus not due to progressive radiation damage to the insulin but to interaction of the insulin with some serum factor, either pre-existing or liberated or activated on incubation at 35°. To investigate this, experiments such as those shown

in Fig. 2 were carried out. Pre-treatment of the serum at 35° (Fig. 2(b)) rather than at 4° (Fig. 2(a)) considerably increases the amount of α_2-activity produced on subsequent incubation with [^{131}I]insulin at 4°, though this is still not as great as that obtained when the labelled insulin is present also during incubation at 35° (Fig. 2(c)). The appearance of α_2-activity is thus not entirely (though perhaps partly) due to a pre-existing factor in serum which reacts with [^{131}I]insulin, since incubation of serum alone increases its capacity for producing α_2-activity.

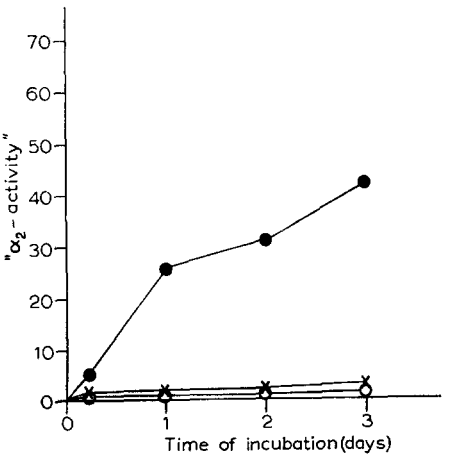

Fig. 1. Effect of time and temperature of incubation on the appearance of "α_2-activity" (expressed as percentage of the total radioactivity in the sample). Temperature of incubation: ●—●, 35°; ×—×, 20°; ○—○, 4°.

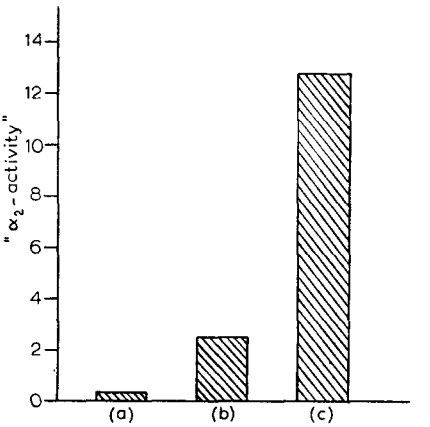

Fig. 2. Effect of pre-incubation of serum on appearance of "α_2-activity" (expressed as percentage of total radioactivity in the sample). (a), Serum incubated at 4° for 1 day, then with [^{131}I]insulin at 4° for 1 day. (b), Serum incubated at 35° for 1 day, then with [^{131}I]insulin at 4° for 1 day. (c), Serum incubated with [^{131}I]-insulin at 35° for 1 day, then at 4° for 1 day.

Furthermore, the fact that the α_2-activities of (b) and (c) (Fig. 2) are different, suggests that the serum factor produced on incubation at 35° does not react rapidly with the [^{131}I]insulin, and that the rate of reaction is faster at 35° than at 4°. The overall rate of appearance of α_2-activity when serum is incubated with [^{131}I]insulin (Fig. 1) may thus be the resultant of two comparatively slow temperature-dependent processes, the production of some factor in the serum and the reaction of that factor with the insulin.

There are several possibilities as to the nature of the α_2-radioactivity. It might be due to insulin or to some radioactive degradation product of insulin bound either to α_2-globulin or to some other serum fraction with which it forms a complex having an electrophoretic mobility similar to that of α_2-globulin. It is apparently not an easily dissociable complex involving unchanged insulin; the α_2-activity, when eluted from one paper and re-run on another after the addition of unlabelled insulin, migrated again to the α_2-globulin position. If there had been any exchange between free (inactive) and bound (radioactive) insulin, radioactivity would be expected at the origin but none was detected.

Inorganic [^{131}I]iodide released from the labelled insulin has also been excluded

since serum incubated for several days with [^{131}I]iodide shows only a trace of radioactivity in the albumin and none in any other fraction.

Further work is in progress on the nature of the α_2-activity and of the processes causing it, including the possibility of bacterial action.

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The influence of sulfur compounds on molybdenum toxicity in *Aspergillus niger*

From investigations carried out with several organisms, it has become evident that the toxic effects of molybdenum can be counteracted, in many cases, by sulfur-containing compounds, such as inorganic sulfate, or the amino acids cysteine and methionine. GRAY AND DANIEL[1] showed that methionine, at very high dietary levels, suppressed the growth-inhibitory effect of molybdenum in rats. Later, VAN REEN AND WILLIAMS[2] demonstrated that inorganic sulfate and cysteine had similar beneficial effects. In *Neurospora crassa*, SIVARAMA SASTRY *et al.*[3] found that molybdenum toxicity depressed the biosynthesis of methionine and that, at levels of molybdenum causing around 50% inhibition of growth, methionine completely prevented the growth suppression when supplemented at the low concentration of 5 μg/ml in the basal medium. Since this was the first demonstration of a direct interference with sulfur amino acid metabolism by molybdenum in microorganisms, it was thought of interest to explore whether this was a general phenomenon. The present communication deals with the influence of several sulfur compounds on molybdenum toxicity in the mold *Aspergillus niger*.