## Starch–Agar-Gel Electrophoresis of Tuberculoproteins from Heated Culture Filtrates

IN order to improve the specificity of a bovine tuberculin purified protein derivative, precipitated with trichloracetic acid from heated culture filtrates[1], we decided to investigate its protein composition[2], and in this connexion we experimented with zone electrophoresis on paper-strips. Now this procedure has hitherto not been very successful[3-5], and in spite of our own numerous experiments with various procedures and methods we did not obtain anything other than unusable pictures of 'comets' or streaks. (This is in contrast with the more satisfying results obtained by others with non-heated tuberculins[4,5].) Smithies introduced starch gel electrophoresis which seems to be of great value in determining the ultra-structure of serum-protein patterns[6,7], the composition of toxins[8] and of cerebrospinal fluid[9]. Sorkin *et al.*[10] made preparative separations of some tuberculoprotein preparations from unheated culture filtrates by electrophoresis in starch gels, followed by transfer into glass. Our own investigations on this starch-gel electrophoresis led to the following method.

In order to prevent premature desiccating of the gels and to avoid the necessity of protecting them by placing covers and/or mineral oils on the surface and still to maintain a satisfactory consistency, we used 10 per cent w/v 'Merck Amylum Solubile' (*A.R.*) plus 0·27 per cent w/v 'Difco Bacto' agar. The agar was at first weakened for about 30 min. or longer in the buffer solution consisting of 15 gm./l. boric acid, 0·75 gm./l. bi-sodium salt of ethylenediamine tetraacetic acid and 2·5 mgm./l. 'Tween 80', brought up to $p$H 7·5 by adding about 6 gm./l. *tris* hydroxymethyl aminomethane. The mixture was then boiled until the agar was dissolved. After some time of cooling the solution, the starch was added, suspended, heated with continuous stirring and finally boiled for a few seconds. The liquid gel was poured out in plastic ('Perspex') trays to form layers of 240 mm. × 60 mm. × 3 mm., the trays having been placed in an electrophoresis apparatus with platinum electrodes. The electrode vessels and bridge vessels were filled with the above-mentioned buffer, only without 'Tween 80', and with sodium hydroxide instead of *tris* buffer. Thick filter-paper strips formed the connexion between bridge vessels and tray. After about 20 min. we made an incision, 8 cm. from the anodic side of the tray, with a thin blade and allowed 0·05–0·07 ml. of a protein solution of about 60 mgm./ml. to drip down slowly into the incision, even when it was scarcely visible on the surface. Immediately afterwards the gels were subjected to electrophoresis at room temperature for 17 hr. at 6 V./cm., then left to stain with Bayers 'Amidoschwarz 10 *B*' (2 gm./l. solution of methanol/water/acetic acid, 45 : 45 : 10) for 15 min., and washed with the same solution over a period of 1 hr., and repeated about 5 times with fresh solution. Fig. 1 shows the patterns of our bovine purified protein derivative tuberculin, solved from the lyophilized state by dialysing against a 0·25 per cent phenolic diluted buffer of $p$H 7 until the required protein concentration was reached; the '*C*'-like protein fraction, prepared from the same purified protein derivative, fundamentally following Seibert's method[11] and the '*A* + *B*'-like proteins, prepared from the (purified protein derivative minus '*C*') remainder.

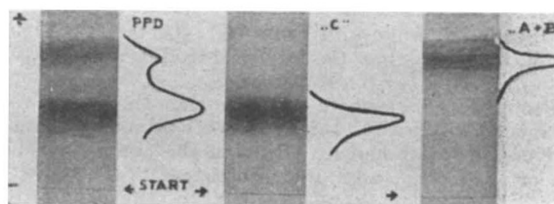


Fig. 1. The curves were obtained from colour diapositives of the patterns by photoelectric scanning

Full details of these preparations will be described in a publication elsewhere.

It appears from the purified protein derivative diagram that the proportion '*A* + *B*'/'*C*', calculated from planimetric measurement, is about a third, which is in accordance with the quantities of the chemically separated fractions. The '*C*'-like protein, its molecular weight probably being twice as great as that of the unheated '*C*'[12], is the slower one, in this respect quite the reverse of the unheated '*C*' in the Tiselius picture[2]. Furthermore, our '*C*'-like protein shows a greater tendency towards tailing than the '*A* + *B*'-like fraction.

These last two proteins appear to move closely together. $p$H's higher than 7·5 tend progressively to suppress separation while the lower ones tend to sharpen the zones but increase adsorption to the gel substance. Our results point to the combined effects of electric charge and particle size in starch-gel electrophoresis[13].

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## Effect of Pituitary Growth Hormone *in vivo* on the Phosphorus/Oxygen Ratios of Isolated Rat Liver Mitochondria

RECENT reports tending to localize the site of action of thyroxine in uncoupling oxidative phosphorylation at the level of the mitochondrial membrane[1,2] have served to emphasize the need for similar studies on possible sites of action of anterior pituitary growth hormone, a substance which produces generalized metabolic changes even more striking than those found with thyroxine. The present communication reports some results concerning the effect of the hormone on oxidative phosphorylation, using mitochondria isolated from the livers of normal rats or rats treated with growth hormone.

Rats were injected 4 hr. before killing either with 1 mgm. purified beef growth hormone or with 1–5

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