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BEHAVIOR OF THE SERUM PROTEINS IN STARCH - AGAR - GEL ELECTROPHORESIS

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SUMMARY

Behavior of the serum proteins of human and mule have been studied in starch-agar-gel electrophoresis, using progressively increasing concentrations of agar in starch-gel. The smallest amount of added, was 0.7% and the highest amount was 2.0% of the total weight of the starch. The resolution of the serum proteins are altered considerably and this alteration is increased with the increase in percentage of agar in starch-agar-gel system the dye affinity of the starch-gel is also reduced considerably by the addition of a small amount of agar to the starch-gel.

INTRODUCTION

The technique of starch-gel electrophoresis suggested by SMITHIES 1,2 has been found to give a higher resolution of the serum proteins, than other means of electrophoresis. This is attributed to the molecular dimemensions of the starch-gel, which apparently reaches the size of some of the proteins involved. When very small amount of agar is added to the starch, a change in the distribution of the serum proteins is noticed. The degree of this alteration depends on the concentration of the agar added.

The work presented here, was conducted with the purpose to find out, if the combination of starch and agar would give a higher resolution of the serum proteins, particularly of albumin and the gamma-globulin.

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Although the results point to a further reduction in resolution of the serum proteins, in general, it may contribute to the understanding of the physico-chemical behavior of proteins in gel systems. Human and mule sera were used, because at the time these experiments were being carried out, the sera of these two species were available.

MATERIALS AND METHODS

Preparation of agar

Difco agar, 30 gm/400 ml of distilled water was heated until melted and the solution was poured into a large cylindrical beaker. When the gels were solidified, it was cut into small cubes of about one centimeter. These cubes were washed with repeated changes of distilled water, for several days until the cubes became completely white. Appropriate concentrations varying from 0.5 to 1.5 gm of agar per 100 ml of distilled water were made from this, after the exact concentration of agar was measured by determining its dry weight. Bottles containing 100 ml of the solidified mixture were maintained in refrigerator, until used. If it was desired to keep the agar for a longer period of time, merthiolate, 1/10000 was added, as a preservative.

Preparation of starch-agar-gel

Starch-gel was prepared from Starch Hydrolysed. Seventy four grams of starch (approximately 12.3 gm/100 ml) was added to 600 ml of a borate buffer of PH 8.65 and the ionic strength of 0.03, and the gel was made as recommended 1, and then was poured into the plastic tray. When it was desired to prepare starch-agar-gel, the starch was addad to 500 ml of a buffer of the same composition, as given above, except that it was 1.2 times more concentrated. The starch and buffer mixture was heated with constant shaking, and when the temperature reached about 40 to 45 C, the bottle containing 100 ml of the desired concentration of agar in distilled water, which had been melted by heating it in boiling bath, and had been left to cool down to reach the temperature of the starch buffer mixture, was then added to the mixture. The heating and shaking was continued until a homogeneous gel was formed. In order to have the same final concentration of solid materials for both starch-gel and starch-agar-gel, the amount of agar present in the mixture was deduced from the initial amount of the starch which was to be weighed. In order to be ensured of the similarity between

the PH's for both kinds of gels, their PH's were determined just before they began to solidify, and was usually found to be similar. In order to minimize the sources of error which would bring differences in the serum pattern, not related to the gel composition, it was sometimes necessary to cast both kinds of gel in the same tray; one gel being composed of starch alone and the other containing starch and agar of the desired concentration, each occupying exactly half of the tray, having four slots for sample insersion.

Vertical electrophoresis 2 with a voltage gradient of approximately 4 V/cm across the gel was applied, for a period of 16 hours. Electrophoresis was performed at room temperature, using Elphor apparatus as the power source.

Photographs were taken on Kodak, panatomic X films, using a Leica, III-f camera.

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RESULTS

Fig. 1 is a schematic representation of the electrophoretic pattern of three different individual human sera. The letters a, b, and c refer to the patterns obtained in a starch-gel containing 0.5, 1.0 and 1.5 gm of agar and 73.5, 73.0 and 72.5 gm of starch in every 600 ml of buffer solution, respectively. The pattern of the serum, when starch-gel alone is used, is also demonstrated, for comparison. When the amount of agar added, is only 0.5 gm and the amount of starch 73.5 gm (approximately 0.7% of agar in starch), the change in the pattern is little; the albumin band is less spread in starch-agar-gel, and there seems to be a somewhat less entry of gammaglobulin towards the cathod from the inserison slot. Increasing the amount of agar, from 0.5 to 1.0 gm (1.37% of agar in starch), the pre-albumins disappear and the albumin band becomes even more compact. The amount of gammaglobulin entering the block, is considerably reduced. When the amount of agar is increased to 1.5 gm (approximately 2.5% of agar in starch), many protein fractions disappear.

Fig. 2 is representative pattern of the serum of a mule, run in the conditions, as stated for human sera. The serum of this mule has previously been tested on paper and agar electrophoresis 3 and it was found that its albumin band behaved as being composed of two fractions. It was thought that it would be interesting to see whether a similar behavior could also be seen when run on starch-gel and starch-agar gel electrophoresis. When

the serum was run on starch-gel alone, the albumin band did not show any separation, but the addition of agar to the starch-gel, caused the reappearance of the "double band" of the albumin. The increase in the compactness of the albumin band, and the disappearance of gamma-globulin with the increase in the percentage of agar in starch-gel, was similar to that of human serum. An increase in the resolution of the mule serum was noticed, however, with the addition of 0.5 and 1.0 gm agar to 73.5 and 73.0 gm of starch, respectively. Increasing the amount of agar to 1.5 gm, for 72.5 gm of starch, caused the disappearance of the protein bands, specially the post-albumins.

Using starch-gel of various concentrations, as it was indicated above, the proteins, as well as the gel, lost their dye affinity considerably.

DISCUSSION

The smallest percentage of agar added to the starch-gel, was approximately 0.7% Behavior of the serum proteins in this concentration seems to have changed only slightly. The pre-albumins disappeared, and there also seemed to be less entery of gamma-globulin in the direction of cathod (Fig. 1). The other fractions remained unchanged. As the percentage of agar was raised to approximately 1.3% further changes were observed. Besides the pre-albumins, some of the post-albumins also disappered, and the amount of gamma-globulin was further reduced. Increasing the amount of agar to as much as 2.0% of starch, still several other fractions disappeared. The beta-and the slow-alpha-2 globulins, however, were only slightly altered. The abnormal mule serum albumin behaved as a "double band" in starch-agar-gel, as it also did on paper electrophoresis, but not in starch-gel alone. When the serum of this particular mule was diluted with physiological saline, the double band also apperared in starch-gel alone 3.

SMITHIES 1, has attributed the greater resolution of the serum proteins in starch-gel to the sieve effect of pores in the gel, which are apparently of a size comparable to the protein molecular dimensions. RAYMOND AND NAKAMICHI 4 have recently investigated the relationship between the pore size and the mobility of the proteins, using synthetic gels. Within a certain range of concentration of the gel, no effect of pore size could be demonstrated by them.

The addition of agar to starch-gel, effects the relative mobility of the various fractions, and also the resolution of the proteins are considerably altered.

SMITHIES 5 has recently given a mathematical account of the migration of B chain of insulin and alpha and beta chains of haptoglobin in progressively increasing amounts of starch in starch gel and has concluded that the migration of any single protein is proportional to the reciprocal of the starch concentration in starch gel. He has further elucidated the point that the retardation coefficient of a given ion is dependent on the size of the ion but is independent of its charge and the duration of electrophoresis.

It seems that in a system having small amounts of agar in starch gel would considerably alter the physico-chemical properties of the gel. Such a system is so much complex, however, that any conclusion regarding its be havior would await further analysis, using purified proteins of known molecular size and electrical charge. From the standpoint of the experimental results, given, it can presumably be said that as the agar concentration increase in the starch-agar-gel, the gel system no longer behaves as it does, when starch gel alone is employed, and that its properties, at least in some respects, would approach that of agar gel system. The fact that the mule serum with double albumin band, when undiluted, behaves similarly in agar paper and starch-agar-gel electrophoresis and not in starch-gel alone, might point towards this direction. Increasing the starch concentration in starch-gel, would only affect the relative mobility of the ion species, and not the resolution of at least certain proteins, as shown by SMITHIES 5, within certain concentration range tested. Increasing the concentration of agar in starch-agar-gel, of even smaller range, as indicated above, would alter the resolution as well as the mobility, depending on the protein nature.

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REFERENCES

1 O. SMITHIES, Biochem. J., 61 (1955) 629.
2 O. SMITHIES, Biochem. J., 71 (1959) 121.

3 A. AMIN, and K. D. SHAMLOO, Canadian J. Biochem. & Physiol. (in press)
4 S. RAYMOND and M. NAKAMICHI, Anal. Biochem. 3 (1962) 23.
5 O. SMITHIES, Arch. Biochem. Biophys., Suppl. 1 (1962) 125.

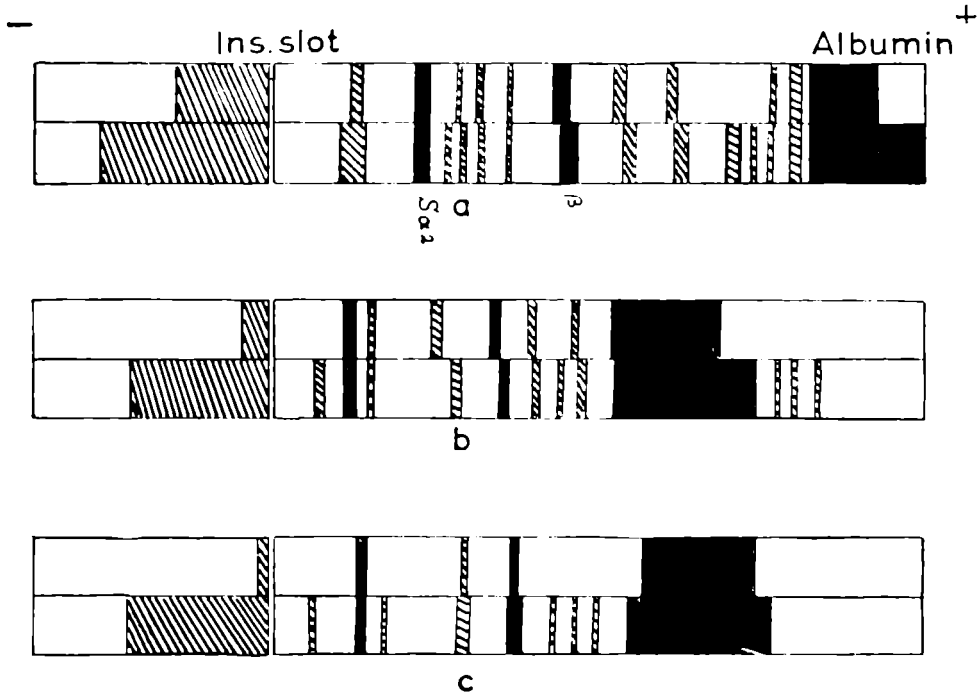
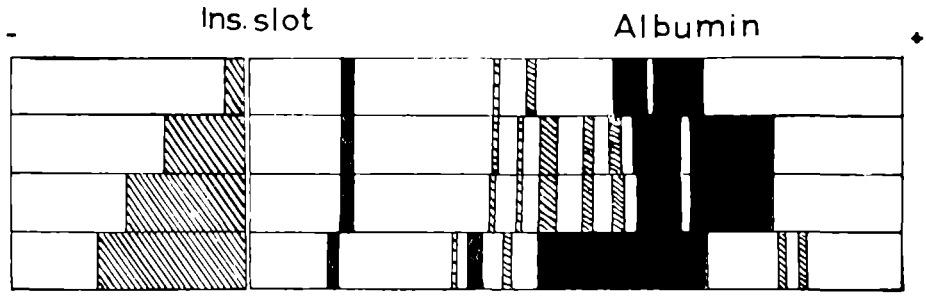


Fig. 1. A schematic representation of starch-agar-gel electrophresis of sera of three different human individuals. Letters a, b and c refer to the patterns obtained in starch-gel containing in starch-gel containing 0.5, 1.0 and 1.5 gm of agar added to 73.5, 73.0 and 72.5 gm of starch for every 600 ml of buffer, respectively. The lower ones in each, refer to the patterns run in starch-gel alone.



The Fig. 2. A schematic representation of starch-agar-gel electrophoresis of a mule serum.

The patterns from top to bottom are in mixtures of agar starch containing 1.5, 1.0 and 0.5 gm of agar in 72.5, 73.0 and 72.5 gm of starch in every 600 ml of buffer. The one at the extreme bottom is run in starch-gel, alone.

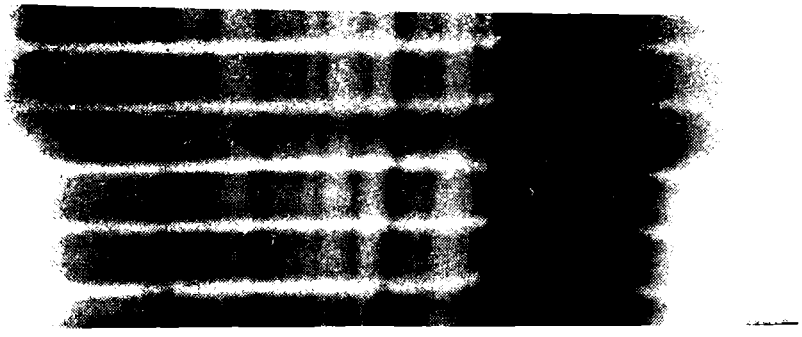


Fig. 3. Aphotograph of starch-gel (the top three) and starch-agar-gel (the bottom three) of an individual human serum, run simultaneously on the same block.

The amount of agar added, was 0.5 gm to 73.5 gm of starch, for every 600 ml of buffer.