

1962

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### Recommended Citation

Flickinger, R. A. (1962) "Immunological Comparison of Rat Liver Tumors with Normal Rat Livers," *Proceedings of the Iowa Academy of Science*: Vol. 69: No. 1 , Article 92.

Available at: <https://scholarworks.uni.edu/pias/vol69/iss1/92>

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## Immunological Comparison of Rat Liver Tumors with Normal Rat Livers<sup>1</sup>

R. A. FLICKINGER<sup>2</sup>

*Abstract.* Antisera were developed against centrifugal supernates and crude ribonucleoprotein fractions of normal rat livers and liver tumors. These antisera were tested with the homologous fractions of both the rat liver tumor and normal rat liver in agar-diffusion reactions. In no case was a definite reaction of nonidentity observed although a liver tumor antigen spur was sometimes observed in the reactions that utilized antisera directed against centrifugal supernates of liver tumors. The results indicate a qualitative similarity of a number of antigens derived from liver tumors and normal rat livers.

Weiler (1956), using fluorescein-labelled antibodies, found that rat hepatomas induced by feeding 4-dimethylaminoazobenzene lack an organ-specific antigen located on cytoplasmic particles of normal rat liver. This finding remained of interest even though Hughes (1958) discovered that fluorescein-labelled globulin of normal rabbit serum from unimmunized rabbits did not stain certain islands of liver parenchyma cells in rats being fed an azo dye, although normal liver parenchyma cells did stain, thus suggesting a loss of protein that ordinarily binds normal rabbit globulin.

Sorof, et al. (1951) have demonstrated electrophoretically that in early stages of azo dye carcinogenesis, soluble "h" proteins of rat liver bind a large share of the azo dye, but in rat liver tumors the "h" proteins are almost entirely absent (1951). These data would appear to lend some support to the hypothesis that the azo dyes are carcinogenic due to their property of deleting specific proteins in rat liver which normally have a growth-controlling function (Miller and Miller, 1947). In the present investigation a further comparison of azo-dye-induced rat liver tumors and normal rat livers was made by immunological procedures. Recently Perlman et al. (1959), and D'Amelio and Perlmann (1960) have characterized immunologically, antigens of the cell sap, mitochondria and microsomes of normal rat liver, by agar-diffusion reactions and their work provides a critical picture of some of the numerous antigens of rat liver.

### MATERIALS AND METHODS

*Preparation of Antisens.* Liver tumors were induced by feed-

<sup>1</sup> This research was supported by a grant (6139) from the National Science Foundation.

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ing the rats 3-methyl-4-dimethylaminoazobenzene for three months at a 0.06% level in a synthetic diet (Gelboin et al., 1958), followed by 1-2 months of feeding rat food pellets. Antisera were developed to centrifugal supernates (2,500 g) and crude ribonucleoprotein fractions (RNP). Homogenates were prepared by blending equal amounts of liver tissue and 0.85% NaCl in the Waring Blender, followed by homogenization with an all-glass tissue grinder. The livers were cut into many small pieces and washed with several changes of cold saline in the refrigerator, over a two-hour period, before they were placed in the blender. Centrifugal supernates were obtained by centrifuging such homogenates for 15 minutes at 2,500 g. The ribonucleoprotein fractions were obtained from centrifugal supernates by lowering the pH to 4.5, washing the precipitate with a pH 4.5 saline solution, and then raising the pH to 7.2 to solubilize RNP from the precipitate. Test antigen preparations were adjusted to a concentration of 1% protein after nitrogen determinations with Nessler's reagent.

*Preparation of Antisera.* Emulsions of 0.5 ml of Freund's adjuvant and 0.5 ml of antigen were injected intramuscularly, at different sites, three times a week for one month; the rabbits were rested three weeks and then bled. The monthly injection series was repeated and the final bleeding was performed three weeks later. The antisera developed against the centrifugal supernates and RNAP fractions of normal and tumorous livers reacted with the homologous antigens, diluted to a 1% protein concentration, in precipitin reactions at antisera dilutions of 1/512. The various antisera were reacted with the soluble fractions of liver tumors and normal rat livers at a 1% protein concentration in agar-plate diffusion reactions (Björklund, 1952; Ouchterlony, 1948).

## RESULTS

*Agar-Diffusion Reactions.* All of the antisera were tested with the different fractions of liver tumor and normal liver in Ouchterlony agar-diffusion reactions, but in no case was a definite difference noted between the antigenic composition of the fractions of liver tumor and normal liver. Such a difference would have been indicated by the crossing over of precipitate lines in the "reaction of non-identity" of Björklund (1952). The antiserum obtained by immunization with normal liver centrifugal supernate fraction + Freund's adjuvant was reacted with tumor and normal liver RNAP and the results are illustrated in Fig. 1.

It is evident that all antigens showed reactions of identity, i. e., the precipitate lines did not cross over. The reaction between an antiserum to a tumor centrifugal supernate and the RNAP fractions of liver tumors and normal livers is represented in Fig.

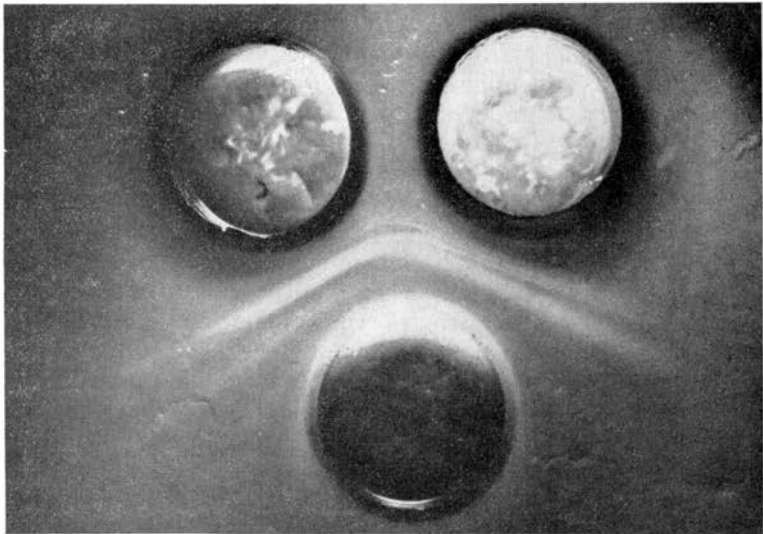


Figure 1. Anti-normal liver centrifugal supernate serum (lower well) reacted against liver tumor RNAP (upper left well) and normal liver RNAP (upper right well).

2. This particular antiserum produced an antigen "spur" in the reaction with the liver tumor RNAP fraction and seems to be characteristic of the "reaction of partial identity" (Björklund, 1952).

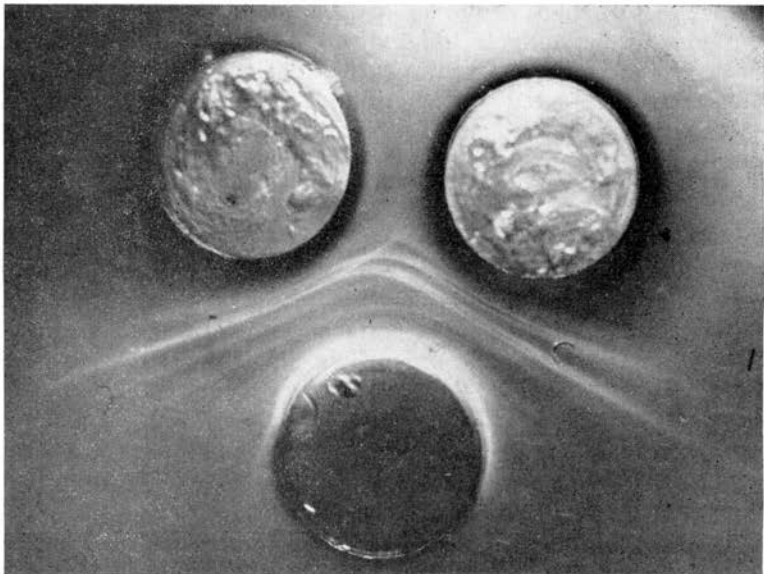


Figure 2. Anti-liver tumor centrifugal supernate serum (lower well) reacted against liver tumor RNAP (upper left well) and normal liver RNAP (upper right well).

### DISCUSSION

The results of the various agar-diffusion reactions provide evidence indicating the qualitative similarity of a number of antigens in the corresponding fractions of liver tumors and normal livers. Further work will be necessary to assess the significance of "spur" formation (reaction of partial identity, Björklund, 1952) in the reactions of some of the antisera to liver tumor centrifugal supernate with the liver tumor RNAP fraction, but not the homologous fraction from normal livers. This may indicate a configuration change in one of the antigens of the normal rat liver, but speculation at the present time would be premature.

With the advent of rapid cellular proliferation in the tumors, there is undoubtedly a quantitative variation of certain antigens in comparison with the normal rat liver, but this was not reflected for the fractions used in this study by the agar-diffusion technique, which is primarily a qualitative method. There are also differences in antigens between individual rats, which would be revealed by transplantation studies, but not by the method used in this investigation.

Although the liver was cut into numerous small fragments and washed thoroughly with saline in order to remove serum proteins, a number of the antigen-antibody precipitates showed reactions of identity with rat serum. This may be partially attributed to serum proteins not removed by washing; but, since the liver cells synthesize serum proteins, the reactions are likely due to definitive serum proteins within the liver cells.

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