Preneoplastic Antigen as a Marker for Endoplasmic Reticulum of Putative Premalignant Hepatocytes during Liver Carcinogenesis

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SUMMARY

Further study of the subcellular localization of a preneoplastic antigen in hyperplastic liver nodules and primary hepatocellular carcinoma induced in rats by acetylaminofluorene has been pursued. Antiserum against four subcellular fractions, cytosol, smooth and rough endoplasmic reticulum, and free polysomes, were obtained in rabbits and were used to assay for the presence of the antigen by immunodiffusion. The preneoplastic antigen, Antigen 1, appearing as a sharp precipitin line, is located predominantly, if not exclusively, in the smooth endoplasmic reticulum fraction of hyperplastic nodules and hepatomas. A second antigen, Antigen 2, appears in the smooth endoplasmic reticulum of nodules after 13 weeks of acetylaminofluorene feeding but gradually disappears on discontinuation of exposure to the carcinogen after 20 weeks. This antigen, appearing as a more diffuse precipitin line, becomes demonstrable in rough endoplasmic reticulum as well but only after stripping off the ribosomes. The presence and distribution of preneoplastic antigen during carcinogenesis as revealed by immunodiffusion was similar when a more sensitive assay, microcomplement fixation, was used. The preneoplastic antigen appears to be a potentially useful marker for alterations in the smooth endoplasmic reticulum that may be related to the development of liver cancer.

INTRODUCTION

The development of cancer in many organs and tissues is preceded by 1 or more new cell populations that differ from the original target cells and that seem to be involved in the cell evolution to malignant neoplasia (9, 13, 25). The hyperplastic nodules in rat liver induced by various chemical carcinogens are believed to be the forerunners of carcinoma of the liver in experimental animals (8, 10, 11, 24). An apparently similar type of hyperplastic lesion has also been suggested as a precursor for liver cancer in human cirrhosis (6, 15). The study of the possible function of such lesions in carcinogenesis has been seriously hampered by the lack of positive markers that identify them and permit in-depth analysis. Tumor-associated antigens in hepatomas induced by chemical carcinogens were reported by several laboratories (2, 3, 14, 16, 17). The nature and characteristics of these antigens have been reviewed in detail recently (1, 4). Whether these or similar tumor-associated antigens are also present in the premalignant hepatocytes during liver carcinogenesis is not known. Another important marker for hepatocellular carcinoma, α-fetoprotein, has been found to be elevated during liver carcinogenesis induced by 2-AAF (5). Recently, we found (20, 21) in hyperplastic nodules of liver induced by 2-AAF or ethionine an apparently new highly reproducible antigen, tentatively called PN antigen. It appears in the earliest new hepatocyte populations, persists in the focal hyperplastic liver cell populations throughout the carcinogenic process, and is also present in primary hepatocellular carcinoma induced by 2-AAF, ethionine, 3'-methyl-4-dimethylaminoazobenzene, dimethylnitrosamine, and diethylnitrosamine. Thus, it appears to have a close relationship to the carcinogenic process in the liver. In previous studies, antiserum to PN antigen was induced in rabbits with crude preparations of PMS from hyperplastic nodules (20, 21). Immunodiffusion assays indicated that the PN antigen is probably "microsomal" in distribution. As part of an attempt to understand the structural and functional properties of PN antigen, it is important to establish the subcellular localization of this antigen. In this paper we extend our previous findings and report the subcellular localization of PN antigen in rat liver hyperplastic nodules induced by 2-AAF. Four fractions of PMS, cytosol, SER, RER, and free polysomes were used to prepare appropriate antisera, and the presence of PN antigen in each component was studied. The distribution of the antigen is the subject of this communication.

MATERIALS AND METHODS

Treatment of Animals

Male Fischer rats (130 to 150 g) were obtained from Charles River Breeding Laboratories, Wilmington, Mass.

* The abbreviations used are: 2-AAF, 2-acetylaminofluorene; PN, preneoplastic; PMS, postmitochondrial supernatant; SER, smooth endoplasmic reticulum; RER, rough endoplasmic reticulum; TKM, 50 mm Tris-HCI, 25 mm KCl, 5 mm MgCl2, 6H2O, pH 7.5; RERst, stripped rough endoplasmic reticulum; SERst, stripped smooth endoplasmic reticulum.

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These animals were fed the basal diet (7) for 1 week before treatment with carcinogen. The experimental animals were continuously fed the basal diet containing 0.02% 2-AAF (Bio-Serv Inc., Frenchtown, N. J.) for 18 weeks and then switched to the basal diet. Under these feeding conditions, very small hyperplastic nodules could usually be seen at 3 weeks, and discrete nodules of increasing size were developed after 13 weeks. The control animals were fed continuously the basal diet only.

Liver Fractionation

Both carcinogen-fed and control rats were killed by decapitation, and livers were perfused in situ with sterile cold 0.9% NaCl solution. Hyperplastic nodules were removed from the livers of rats fed the 2-AAF diet for 13 to 18 weeks. Whole livers or hyperplastic nodules were pooled, minced, and homogenized with 2.5 volumes of ice-cold 0.25 m sucrose:TKM. The homogenate was centrifuged at 800 \( \times \) g for 15 min, and the nuclear pellet was discarded. The PMS was obtained by 2 cycles of centrifugation at 2,000 \( \times \) g and 15,900 \( \times \) g for 15 min in a Sorvall SS-34 rotor. After each centrifugation, lipid accumulations were removed from the surface of the supernatant and the pellet was discarded. The cytosol fraction was prepared by centrifuging the PMS at 105,000 \( \times \) g for 1 hr in a 50 Ti rotor in a Beckman Model L5-65 ultracentrifuge. All procedures were carried out at 0–4°C unless otherwise noted.

Separation of Endoplasmic Reticulum and Polysomes

Endoplasmic reticulum and free polysomes were prepared from the PMS according to the procedures of Sunshine et al. (27). The PMS was mixed with 1.71 volumes of 2 m sucrose:TKM, making a final concentration of 1.3 m sucrose:TKM. For separation of the smooth and rough membrane subfractions, 4.5 ml of the PMS in 1.3 m sucrose:TKM were layered onto 3.5 ml of 2 m sucrose:TKM, and 2 ml of 0.25 m sucrose:TKM was layered on the top. After centrifugation at 105,000 \( \times \) g for 4 hr in a 50 Ti rotor in a Beckman Model L5-65 ultracentrifuge, the rough membrane fraction (RER) was localized at the interface between the 1.3 and 2 m sucrose layers. The smooth membrane (SER) remains at the interface between the 0.25 and 1.3 m sucrose layers, and the “free” polysome fraction sediments through the 2 m sucrose layer and forms a glassy pellet at the bottom of the tube.

Electron Microscopy

Purity of membrane preparations was examined by electron microscopy. Rough and smooth membrane fractions were carefully aspirated from the sucrose gradient, diluted with 3 volumes of TKM buffer without sucrose (pH 7.5), and pelleted by centrifugation at 105,000 \( \times \) g for 1 hr. The pellets were fixed in 1.25% glutaraldehyde in 0.1 m sodium cacodylate buffer containing 7.5% sucrose, pH 7.4, for 1 hr at 0°C and washed once with 0.1 m sodium cacodylate:sucrose buffer, pH 7.4. The tissues were kept in this buffer at 4°C overnight and postfixed in 1% OsO\(_4\) in 0.1 m sodium cacodylate:sucrose buffer, pH 7.4, for 1 hr at 0°C. After fixation, the tissues were dehydrated in an ascending series of alcohol concentrations followed by propylene oxide; they were subsequently embedded in Epon:Araldite. Thin sections approximately 50 to 100 Å thick were cut with a Porter Blum ultramicrotome. The sections were positively stained with lead citrate (Reynolds I) for 20 to 30 min followed by uranyl acetate for 15 min. Examination was performed with a Philips EM-300 electron microscope.

Immunization Schedule

RER, SER, polysome, and cytosol prepared as described above were used to immunize New Zealand White rabbits as previously reported (28, 29). Briefly, aliquots of a sample containing 1 mg of protein were suspended in an equal volume of complete Freund’s adjuvant (Difco Laboratories, Detroit, Mich.) Four injections were given intradermally to rabbits on the 1st, 8th, 15th, and 22nd days. A s.c. booster injection was given 1 week later, and serum was obtained 1 week after that. Protein was determined by the method of Lowry et al. (19).

Analysis of Sera

Microcomplement Fixation. Titrations of antisera against hyperplastic nodule RER, SER, polysome, and cytosol were performed according to the method of Levine and Van Vunakis (18).

Immunodiffusion. The antisera against hyperplastic nodule RER, SER, polysome, and cytosol were individually absorbed with a PMS from normal rat liver. At least 4 to 6 precipitin lines were observed prior to the absorption. The control experiments were performed by absorbing these antisera with PMS from hyperplastic nodules. The absorptions were carried out by incubating aliquots of antisera with varying amounts of PMS (v/v) at 37°C for 2 hr followed by incubation at 4°C for 16 hr. The aggregated antigen-antibody complexes were removed by centrifugation at 4000 rpm for 15 min in an IEC refrigerated centrifuge (PR-6000).

The supernatants were assayed for the completeness of absorption in the Ouchterlony immunodiffusion plate (22) following reactions with the PMS of hyperplastic nodules. The absorbed antisera, at the absorption ratio (v/v) of antigen to antiserum that gives the least number of precipitin lines, was chosen to assay for the presence of specific antigen in each subcellular component of hyperplastic nodules. The precipitin lines were usually visible at 72 hr after incubation in a moisture chamber at room temperature.

RESULTS

Electron Microscope Examinations. Typical RER isolated from normal liver and hyperplastic nodule are shown in Figs. 1 and 2. The preparations from both sources are free of major contamination by other organelles, except for a few coated vesicles. RER vesicles from nodules are not seen in as sharp a focus (Fig. 2) as are those from normal liver (Fig. 1). The SER from normal liver (Fig. 3) and from nodules (Fig. 4) have no obvious major contamination by...
free ribosomes and RER. However, a few coated vesicles and lysosomes are occasionally seen.

**Titration of Antisera.** The titrations of rabbit antisera against SER, RER, polysomes, and cytosol from hyperplastic nodules were performed by microcomplement fixation. The dilutions of antisera against each subcellular component were usually greater than 1:600 after the 3rd week of immunization. One week after a booster injection, the dilutions of antisera used were increased to 1:3200, 1:2400, and 1:1200 for SER, RER, and polysomes, respectively. In contrast, only a 1:800 dilution of antisera against nodule cytosol fraction was feasible. A typical antibody titration curve is shown in Chart 1. As can be seen, even at 1:3200 sera dilutions, the complement-fixing capacity is as high as 75%. Practically, the antisera dilution that displays a complement-fixing capacity as high as 85% is used for the subsequent experiments in microcomplement fixation.

**Subcellular Localization of PN Antigen by Immunodiffusion Technique.** The presence of the antigen in each of the 4 subcellular components from hyperplastic nodules was assayed in Ouchterlony immunodiffusion plate using absorbed antisera against each subcellular component of hyperplastic nodules. At least 4 to 6 precipitin lines were observed prior to absorption. At the absorption ratios (antigen:antiserum, v/v) of 1:2 for cytosol, 1:3 for polysome, and 1:4 for RER antisera, the precipitin lines observed were completely absorbed out. The antisera thus absorbed did not react with any subcellular components from normal liver or from hyperplastic nodules. In contrast, at the absorption ratio of 1:4, the absorbed antisera against SER of hyperplastic nodules selectively reacted with the subcellular components of hyperplastic nodules, as judged by the formation of precipitin lines in the immunodiffusion assay. The absorbed nodule SER antisera at this absorption ratio (1:4) produces only a single precipitin line following reaction with the PMS of hyperplastic nodules. Therefore, this absorbed antinodule SER antisera was used in the subsequent studies. Fig. 5 shows the pattern of precipitin lines of nodule subcellular components following reaction with unabsorbed antinodule SER antisera (center well). It is clearly shown that, in addition to some common antigenic components, a sharp precipitin line closer to the antigenic well was observed in SER from early foci after 5 weeks of 2-AAF feeding (Well 2), hyperplastic nodules (Well 3), and hepatoma (Well 4). This precipitin line was not observed in hepatoma RER (Well 5), hepatoma polysome (Well 6), and normal liver SER (Well 1). The identity of these sharp precipitin lines from 3 different sources was also shown in this immunodiffusion plate, since they were fused completely. In addition, a less sharp line in front of the sharp band was observed only in nodule SER (Well 3). In this immunodiffusion plate (Fig. 5), the precipitin lines were stained with Amido black. Unfortunately, those more diffused precipitin lines, including a cluster around the center well, were not well resolved under this staining condition. Therefore, in the subsequent immunodiffusion plates the unstained patterns are shown.

The selective reactivity of absorbed antinodule SER antisem against various subcellular components is shown in Fig. 6. A single precipitin line was observed following reaction with PMS from hyperplastic nodules (Well 3), and double precipitin lines were seen with purified SER from nodules (Well 2). In contrast, no precipitin lines were detectable with SER (Well 4) or PMS (Well 5) from normal liver or RER (Well 6) or polysomes (Well 1) from hyperplastic nodules. The precipitin line between the center well and Well 3 bends slightly at Well 4. This suggests that normal SER may contain some of the same antigen but in much lower concentration. In addition, in separate immunodiffusion plates, no precipitin lines were observed with RER, polysome, and cytosol from normal liver and cytosol from hyperplastic nodules under the same conditions (not shown). It is clearly evident that there are at least 2 antigen components in the nodule SER. The antigen producing a very sharp line (Fig. 6, Well 2), which we call Antigen 1, resembles closely in appearance and position the antigen previously designated PN antigen. The 2nd component, producing a less sharp reaction, is designated Antigen 2. Absorption of antihyperplastic nodule SER antisem with PMS from hyperplastic nodules abolished both reactions.

These results, taken together, indicate that Antigen 1 and Antigen 2 in nodule SER do not represent the precipitin lines obtained due to incomplete absorption.

The possibility that the lack of reactivity of nodule RER with antinodule SER antisem could result from the blockage of antigenic sites by ribosomes was considered. For exploration of this possibility, the attached ribosomes were stripped from nodule RER by the method of Ragland et al. (23), and the Ouchterlony immunodiffusion assay was repeated (Fig. 7). Clearly, a single precipitin line now appeared (Well 4) following the reaction with absorbed antinodule SER antisem (center well). This line completely fused with the Antigen 2 of nodule SER (Well 3). Thus, the hyperplastic nodule RER contains Antigen 2. Moreover, no reaction was detectable with RERst from normal liver (Well 6), normal SER (Well 1), and nodule polysome (Well 5). With nodule PMS, only 1 less sharp precipitin line, Antigen 2, was obtained (Well 2).

Only a single precipitin line was observed following the reaction of nodule RERst with nodule SER antisem. This could be due to either nodule RER containing only 1 reactive component or nodule RER containing both components but with 1 inactivated or removed by the stripping treatment. For testing of these possibilities, both RER and SER from hyperplastic nodule were subjected to the same stripping treatment and assayed by immunodiffusion. Fig. 8

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**Chart 1.** Titration of antiserum against hyperplastic nodule SER with homologous SER as antigen. Antiserum dilution: 1:800 (●), 1:1600 (○), 1:3200 (▲).
shows the results of this experiment. With the SERst (Well 2) and RERst (Well 6), only 1 precipitin line was formed in both cases. This line completely fused with the Antigen 2 of the nodule SER (Well 1). These findings, though demonstrating that stripping treatment either inactivated or removed Antigen 1 and 2, but that the stripping procedure somehow interferes with or removes Antigen 1.

Although we could not find any contamination with plasma membrane of our SER and RER preparations on electron microscopic examination, the possibility that plasma membrane could also contain PN antigen was considered. We isolated the plasma membrane from hyperplastic nodules and normal liver according to the method of Fisher et al. (12). Antiserum against hyperplastic nodule SER did not react with the plasma membrane of either nodules or control liver in an immunodiffusion assay.

**Time of Appearance of PN Antigen during Carcinogenesis.** If the PN antigen is closely related to PN cell populations, it should appear during carcinogenesis at about the time of the 1st appearance of recognizable hyperplastic nodules. This has been examined during carcinogenesis induced by 2-AAF. Very small hyperplastic foci could usually be seen at 3 weeks, and nodules of increasing size were always seen thereafter. PN antigen (Antigen 1) appeared in the liver of animals on the 2-AAF regimen at 3 weeks (see Fig. 8, Well 5, and Fig. 5, Well 2) and thereafter at all time intervals up to 20 weeks, as well as in primary hepatoma (Fig. 8, Well 3, and Fig. 5, Well 4) induced by the same carcinogen. Occasionally, PN antigen (Antigen 1) was detectable by 2 weeks. The PN antigen appearing in the early stage of carcinogenesis and in primary hepatoma appears the same, since only 1 sharp precipitin line, corresponding to antigen 1 in the discrete nodules (between 13 and 20 weeks), was detectable (Fig. 5, Wells 2, 3, and 4). In contrast to PN antigen, Antigen 2 is more evanescent. It appears in nodules about 13 weeks after initiation of the feeding of 2-AAF and persists for the next 7 to 8 weeks but then disappears after the discontinuation of the 2-AAF regimen. No Antigen 2 has been found in PMS or isolated fractions from 2-AAF-induced hepatocellular carcinomas. PN antigen so far has not been found in normal mature liver, regenerating liver, fetal liver, amniotic fluid, adult normal serum, serum from rats with hyperplastic nodules or primary hepatoma, or a variety of normal tissues. In addition, the preimmune rabbit serum did not react with any of the subcellular preparations from normal rat liver or hyperplastic liver nodules. Our repeated attempts to show any identity or antigenic relationship of a-fetoprotein to PN antigen were uniformly negative. Thus, the pattern of appearance of PN antigen is consistent with its association with new focal liver cell populations during carcinogenesis. However, this conclusion is tentative—the development of a much more sensitive assay procedure, now underway, must be completed before this aspect can be further clarified.

**Microcomplement Fixation.** In parallel with the immunodiffusion assay, the immunospecificity of absorbed antinodule SER antiserum was examined by a more sensitive method, microcomplement fixation. The result of this experiment is shown in Chart 2. The absorbed antinodule SER antiserum could recognize the differences between SER from control liver, nodules, and hepatoma. With nodule SER, the complement-fixing capacity was as high as 85%, compared to 67% in hepatoma SER. In contrast, the control liver SER displayed a less than 20% complement-fixing capacity. In addition, the complement-fixing capacities of RER from nodules, hepatoma, and control liver were as low as those from control SER. In immunodiffusion assay we have shown that the lack of reactivity of RER from nodules and hepatoma with antinodule SER antiserum resulted from the blockage of antigenic sites by ribosomes, and the subsequent removal of ribosomes exposed the antigenic binding sites. A similar phenomenon was also observed in complement fixation. Chart 3 shows that the complement-fixing capacities of RERst from nodules and hepatoma were increased up to 55 and 40%, respectively, whereas the capacity of RERst from control liver remained as low as 12%. The complement-fixing capacities of RERst from nodules and hepatoma were not fully equivalent to those from unstripped nodule SER. This finding parallels those in the immunodiffusion assay, namely that only 1 precipitin line was found in RERst from nodules and hepatoma. In immunodiffusion assay we found that Antigen 1 in nodule SERst was either inactivated or removed by stripping. If this is the case, then the complement-fixing capacity of nodule SERst should be significantly affected. As can be seen in Chart 4, the complement-fixing capacity of nodule SERst was greatly affected at the antigen concentration of 0.5 to 1 µg.
in the total reaction mixture. From 21 to 57% of the complement-fixing capacity was reduced in this range of antigen concentration as compared to the unstripped nodule SER. However, at higher protein concentration (2 μg), less reduction of complement-fixing capacity was observed, indicating an alteration of a majority of the antigenic determinants. As a result of the alteration of a majority of antigenic determinants, a lateral shift of the complement-fixing curve was observed. These experiments have been repeated several times, and each time the differences persist.

The appearance of PN antigen associated with new focal liver cell populations during carcinogenesis was also detectable by microcomplement fixation. As early as after 3 weeks of 2-AAF feeding, the complement-fixing capacity had already increased to 40% and it kept increasing with the stage of carcinogenesis, reaching a plateau at 13 to 20 weeks (not shown).

DISCUSSION

It is evident from the results of this study that liver carcinogenesis induced by 2-AAF is associated with the appearance of at least 2 antigenic components, Antigen 1 and Antigen 2, which are either undetected so far in normal liver or detected at only a low level. Both antigens are intimately associated with the SER, and at least 1, Antigen 2, is also associated with the RER of hyperplastic liver nodules. Antigen 1 appears as early as 2 or 3 weeks after carcinogen feeding and persists in both hyperplastic nodules and primary hepatocellular carcinomas induced by 2-AAF. The 2nd antigen, Antigen 2, less sharply defined in immunodiffusion, is evident in nodules after 13 weeks of 2-AAF feeding and gradually fades after 20 weeks. Since this antigen was not found in primary hepatoma, it probably is less intimately related to the carcinogenic process.

Although we could not detect Antigen 1 or 2 in RER of hyperplastic nodules, stripping the RER of its ribosomes did reveal the presence of Antigen 2. Presumably, the protein or its antigenic groups are obscured by the reaction of ribosomes with the membrane. Whether Antigen 1 (PN antigen) is also present in RER could not be determined, since the stripping procedure removes or abolishes the reactivity of PN antigen in SER. However, the similar membrane protein patterns of nodule RER and SER in polyacrylamide gels (J-C. Lin and E. Farber, unpublished data) make it possible that Antigen 1 may also be present in nodule RER. In addition, under the stripping treatment used, the loss of ribosomes was accompanied by the removal of membrane proteins, as revealed in the electrophoretic analysis of polyacrylamide gels (J-C. Lin and E. Farber, unpublished data).

These findings are in agreement with those of Stratman et al. (26) that stripping of rat liver SER and RER reduced by 15% and increased by 30%, respectively, the sulfhydryl groups available for carboxamidemethylation by iodoacetamide.

The essential nature of the PN antigen (Antigen 1) remains unknown. Its occurrence in hyperplastic nodules and hepatoma induced by 1 of 5 chemically different chemical carcinogens (20, 21) makes it probable that it plays some role, either direct or indirect, in the carcinogenic process. The 2nd antigen, Antigen 2, is probably less intimately involved in the development of cancer. Its increasing concentration in nodules with increasing time of exposure to 2-AAF and its disappearance after the carcinogen is removed from the diet suggest that it might be a protein or proteins with either altered antigenicity due to reaction with 2-AAF or with a 2-AAF derivative or that it might be induced by and dependent upon the presence of the carcinogen. The reported presence of carcinogen (2-AAF)-modified antigen components in liver during carcinogenesis (1) is consistent with the former suggestion.

These results, taken together, indicate that the hyperplastic nodules uniformly contain an apparently new antigen in their endoplasmic reticulum. The new antigen appears very early during liver carcinogenesis and seems to persist throughout the carcinogenic process in the hepatocytes of hyperplastic nodules and in the neoplastic hepatocytes in all primary hepatomas. Although no convincing evidence for the appearance of PN antigen in the surrounding nonhyperplastic or nonneoplastic liver parenchyma has been found, it remains possible that it may be present at a low level in normal liver. The PN antigen offers evidence for a unifying link between the hyperplastic cell populations at all time intervals from the very earliest to liver cancer. The characterization of the nature and significance of the PN antigen remains interesting and provocative and awaits its imminent complete purification.

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REFERENCES

Fig. 1. Isolated RER from normal rat liver. The fraction consists predominantly of RER minimally contaminated with a few coated vesicles. Note also the various-sized vesicular formations of RER and ribosomes attached to RER profiles. × 60,000.

Fig. 2. Isolated RER from hyperplastic nodules of rat liver. No other contaminating organelles are seen. Vesicular formations of RER are seen. The profiles of these RER vesicles are not as sharply in focus as are those in Fig. 1, but ribosomes are visible on profiles. × 60,000.
Fig. 3. Isolated SER from normal rat liver. Note the minimal contamination with other organelles except for a few coated vesicles. × 45,000.

Fig. 4. Isolated SER from hyperplastic nodules of rat liver. The preparations are clean except for a few coated vesicles. × 45,000.
Fig. 5. Immunodiffusion assay of unabsorbed antinodule SER antiserum (center well) against various subcellular components from different sources: normal SER (Well 1), SER after 5 weeks of 2-AAF feeding (Well 2), hyperplastic nodule SER (Well 3), hepatoma SER (Well 4), hepatoma RER (Well 5), and hepatoma polysomes (Well 6). The precipitin lines were visible after 72 hr of incubation in a moisture chamber at room temperature. The immunodiffusion plate was soaked in 0.1 M sodium phosphate containing 0.9% NaCl, pH 7.4, for 24 hr and then stained with 0.1% Amido black.

Fig. 6. Immunodiffusion assay of absorbed antiserum against hyperplastic nodule SER. Center well, absorbed antiserum. Absorption was performed as described in the text. The arrangements of antigen in the surrounding wells are as follows: nodule polysome (Well 1), nodule SER (Well 2), nodule PMS (Well 3), normal SER (Well 4), normal PMS (Well 5), and nodule RER (Well 6). In this and the subsequent experiments, all antigens were adjusted to 3 mg of protein per ml.
Fig. 7. Immunodiffusion assay of absorbed antinodule SER antiserum (center well) with normal SER (Well 1), nodule PMS (Well 2), nodule SER (Well 3), nodule RERst (Well 4), nodule polysome (Well 5), and normal liver RERst (Well 6).

Fig. 8. Immunodiffusion assay of absorbed antinodule SER antiserum (center well) with nodule SER (Well 1), nodule SERst (Well 2), hepatoma SER (Well 3), hepatoma RER (Well 4), SER from liver after 3 weeks of 2-AAF feeding (Well 5), and nodule RERst (Well 6).
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