

to contain the natural antibody. Kidney and liver antigens have invariably done so in high dilutions whereas heart and voluntary-muscle extracts have reacted only in the lower dilutions. Saline extracts of normal rabbit tissues reacted notably well with serum from the same individual. When extracts of normal rabbit kidney (fresh) and liver (frozen) were spun at 20,000 rpm or more for 60 minutes little or none of the effective antigen remained in the supernatant liquids, but the resuspended sediments were fully as potent as the whole extracts. Alcoholic extracts of rabbit liver and heart muscle failed to react with sera containing the natural antibody, a finding which contrasts significantly with the good fixation obtained when these antigens were mixed with the sera of rabbits having experimental syphilis. The antigen reacting with the natural antibody is adversely affected by glycerol as also upon standing in saline suspension for several days in the refrigerator or upon heating at 56°C for 30 minutes—characteristics which account in part for the fact that its effects were not observed in control tests of the previous studies.¹

Rabbit sera containing the natural antibody also have the capacity to fix complement in mixture with high dilutions of saline extracts of normal tissues procured from heterologous species (rat, mouse, guinea pig, chicken), and this capacity can be absorbed with normal rabbit-liver extract, though rabbit erythrocytes fail to remove it. Further tests are under way to define more clearly the affinities of the natural antibody and the properties of the substances with which it reacts.

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Distinct Types of Antibodies in the Blood of Rabbits Carrying the Transplanted V2 Carcinoma.

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Rabbits carrying the transplanted V2 carcinoma—a squamous-cell cancer derived originally from a virus-induced papilloma—regularly develop in their blood an antibody capable of neutralizing *in vitro* the papilloma virus (Shope) and capable also of fixing complement in mixture with it.¹ Further studies have now shown that the

¹ Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1940, **71**, 813.

virus-neutralizing and complement-fixing titers of sera procured from rabbits carrying the V2 carcinoma invariably parallel one another, and that the antibody responsible for both reactions can be readily absorbed from the sera upon admixture with the virus. From the findings it appears certain that the antibody is directed against the papilloma virus *per se*, and that it is identical with the antiviral antibody present in the blood of rabbits carrying benign virus-induced papillomas, which is in turn identical with that present in the blood of rabbits injected with purified papilloma virus.² In further serum tests, carried out by the methods used in the serological study of the Brown-Pearce tumor,³ we have recently encountered in the blood of rabbits with the transplanted V2 carcinoma two other types of antibodies, wholly distinct from one another and from the antiviral antibody just described.

When the sera of normal adult rabbits and of rabbits carrying the V2 carcinoma are heated at 56°C for 30 minutes and tested for capacity to fix complement in mixture with unheated 1:20 or 1:40 saline extracts of normal and neoplastic tissues (*e. g.*, normal rabbit liver or kidney, V2 carcinoma, Brown-Pearce carcinoma) complement-fixation takes place in nearly all of the mixtures.* In general the normal sera react in dilutions as high as 1:16 to 1:32 with the normal tissue extracts, and only about half as well with the neoplastic-tissue antigens. On the other hand, sera from rabbits that have carried progressively enlarging V2 carcinomas for some weeks not only react with normal tissue extracts but are vastly more potent in mixture with the homologous antigen, generally fixing 2 units of complement completely in dilutions as high as 1:128 or 1:256 or more in mixture with extracts of the V2 carcinoma. (The sera show no anticomplementary effects in dilutions of 1:2, nor do the antigens when tested simultaneously in double volume.) When heated at 65°C for 30 minutes, the sera of normal rabbits lose completely their capacity to react with either the normal or the neoplastic tissue antigens. The sera of rabbits carrying the V2 carcinoma, by contrast, retain undiminished their capacity to react with the V2 carcinoma antigen, though their ability to react with the normal tissue and heterologous tumor antigens is abolished or markedly diminished.

The heat-resistant antibody capable of fixing complement specifically in mixture with saline extracts of the V2 carcinoma was regular-

² Friedewald, W. F., and Kidd, J. G., *J. Exp. Med.*, 1940, **72**, 531.

³ Kidd, J. G., *J. Exp. Med.*, 1940, **71**, 335, 351.

* For a brief outline of the complement-fixation procedure, see the accompanying paper.⁴

ly encountered in the blood of rabbits that had carried progressively enlarging growths for periods of several weeks (18 rabbits). It was not present in the blood of normal animals (22 rabbits), in that of rabbits recovering from vaccinia, virus-induced fibromatosis, or experimental syphilis (15 rabbits), nor in that of rabbits carrying transplanted cancers of other types. It can be readily differentiated from the natural antibody, which is also present in the blood of normal adult rabbits, both by its specific affinity for the V2 carcinoma antigen and by the fact that it is unaffected by heating at 65°C for 30 minutes, a procedure that inactivates the natural antibody.⁴

The antibody that reacts with extracts of the V2 carcinoma is not the same as that reacting with the papilloma virus, though the 2 occur together in the same sera, often in high titer. Both are heat-resistant, but they can be readily discriminated by their differing affinities. The V2 antibody reacts with extracts of the V2 carcinoma but not with purified suspensions of the papilloma virus, while the antiviral antibody reacts with the virus (and can be selectively absorbed thereby) as not with extracts of the V2 carcinoma.

The experiments have disclosed 3 distinct types of antibodies in the blood of rabbits carrying the V2 carcinoma. Similar studies have been undertaken with other transplanted cancers.

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Excretion of Sulfanylguanidine in Material Drained from Human Biliary Tract.

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Recently a new sulfonamide drug, sulfanylguanidine, has been developed and studied by Marshall and various other workers.¹ Although the solubility of this drug is not markedly different from that of various related compounds, it appears to be absorbed rather slowly from the gastro-intestinal tract. Because in recent studies of the excretion of various dyes by the liver it has been found that marked differences in excretion occur which cannot at present be

⁴ Kidd, J. G., and Friedewald, W. F., *Proc. Soc. Exp. Biol. and Med.*, 1941, **47**, 128.

¹ Marshall, E. K., Jr., Bratton, A. C., White, H. J., and Litchfield, J. T., Jr., *Johns Hopkins Med. Bull.*, 1940, **67**, 163.