TABLE I.

Material	pH	Time, 20°C	Result
1. Eggs in early cleavage stages	*ca.* 6	6 hr	Membranes fragile
2. " " " " "	" 8	3-6 "	Hatched
3. " " " " "	"	1½ "	Membranes fragile
4. Membranes removed from 3	"	6 "	Normal development
5. 4, plus tr. digestion residue	"	12 "	Development stopped

digestive enzymes is not substantiated. While this may still be the case in teleosts, it seems more likely that the injurious action of the egg contents on the embryos was due to digestion products.

The parallelism between the effects of trypsin and normal hatching in *Rana pipiens* is in agreement with the theory that normal hatching is due to a digestive enzyme. It should be pointed out in this connection that the power of trypsin to mimic normal hatching makes it useful in large scale chemical work with early stages of anurans where the presence of the difficultly removable membranes may prove a hindrance to analysis or penetration.

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Effect of Extravasated Antibody upon Antigenicity of Extracts of Virus-Induced Rabbit Papillomas.

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Saline suspensions of virus-induced rabbit papillomas may stimulate the production of specific antiviral antibody when injected intraperitoneally into normal rabbits, as Shope found, even though they contain no pathogenic virus demonstrable by the ordinary test.[1] In experiments of the same sort we found that saline extracts containing infectious papilloma virus in quantity elicited the antibody in much higher titer than extracts in which little or none was present.[2] Other studies already reported from this laboratory have shown that the antibody often extravasates into the large, disorderly papillomas of cottontail rabbits in such quantity as to "mask" the causative virus,[3] and that the antibody can be identified as such in extracts of the growths.[4] With these findings

1 Shope, R. E., *J. Exp. Med.*, 1937, **65**, 219.

2 Kidd, J. G., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 657; *J. Exp. Med.*, 1938, **68**, 703, 725, 737.

3 Kidd, J. G., *J. Exp. Med.*, 1939, **70**, 583.

4 Friedewald, W. F., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 330.

in mind, experiments were undertaken to determine whether the antibody, which accumulates in the papillomas in various amounts depending upon the titer of it in the blood and upon the local vascular conditions determining its extravasation, may not influence the antigenicity of extracts of the growths.

The antigenicity of the papilloma virus, as determined by its capacity to elicit antibody upon intraperitoneal injection into normal rabbits, was found to be markedly reduced when antibody was mixed with it *in vitro* in amounts sufficient to neutralize it. When an excess of antibody was added to a filtrate containing highly infectious virus, the mixture elicited no antibody upon repeated intraperitoneal injections into normal rabbits, although the control mixture containing saline and the same amount of virus (approximately 20,000 infectious doses for each animal) proved highly antigenic.

To procure virus-induced papillomas that were certain to contain the extravasated antibody in quantity, a number of cottontail and domestic rabbits carrying vigorous confluent growths of 2 to 4 weeks' duration were injected intraperitoneally with large quantities of active virus. This greatly raised the titer of serum-antibody but resulted in no new lesions, not only because the rabbits were already partially immune to the virus but because the virus acts only upon epidermis. After the serum-titer had remained for 10 days or more at the high level to which it had been brought the rabbits were killed for material. Although nourished by blood containing much antibody the growths had enlarged steadily—no unexpected finding since circulating antibody is known to be ineffective against virus associated with living papilloma cells.[5] The growths were washed with soap and water and rinsed well to reduce the number of contaminating bacteria, and then cut away with sterile instruments, diced, and saved in 50% glycerol-Locke's solution in the refrigerator. Other rabbits not hyperimmunized but carrying growths of the same duration and derivation, and hence having various but comparatively small amounts of antibody in their blood and presumably little extravasated antibody in the growths, were likewise killed and their papillomas saved. After the growths had been kept in cold glycerol-Locke's for periods up to 2 weeks, 1:10 or 1:20 saline suspensions were made of them. These were centrifugalized lightly and the supernatant liquids, all heavily opalescent but free from gross tissue-debris, were injected intraperitoneally into normal rabbits, the injections being repeated 8 or 9 days later. After a further

[5] Kidd, J. G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64**, 63, 79; Kidd, J. G., *J. Exp. Med.*, 1938, **67**, 551.

interval of 8 to 10 days the rabbits were bled from an ear vein and tested for resistance to the papilloma virus, with tests also of the serum for antibody by means of standardized neutralization and complement-fixation tests.[6]

Materials derived from 10 wild and 11 domestic rabbits were used in 7 comprehensive experiments. The results were consistent and can be summarized together. Extracts of the 7 wild-rabbit papillomas that contained much infectious virus (and by inference little or no free antibody) invariably proved highly antigenic upon intraperitoneal injection into normal rabbits, eliciting antibody in amounts roughly proportional to the quantity of virus injected. Extracts of 3 wild and 6 domestic-rabbit papillomas, which contained in contrast little or no infectious virus demonstrable on test, elicited antibody in small or moderate amounts upon injection into normal rabbits. Extracts of the growths coming from 5 domestic rabbits having notably high serum-antibody titers failed completely to elicit antibody after repeated injections of large amounts (10 cc of 1:10 or 1:20 saline suspensions). The non-antigenic extracts invariably contained considerable amounts of extravasated antibody, as was proved by their capacity to neutralize added virus *in vitro*. The fact was noted incidentally that passively transferred antibody is often responsible for the resistance to the virus that becomes manifest after extracts of papillomas containing much extravasated antibody are injected intraperitoneally into normal rabbits.

The findings would appear to warrant the conclusion that antibody can reduce or abolish the antigenicity of the papilloma virus upon mixture with it *in vitro*, and that sufficient antibody can extravasate into the virus-induced papillomas of wild and domestic rabbits not only to neutralize the virus liberated when the growths are extracted but also to render extracts or suspensions of some growths incapable of eliciting antibody upon injection into normal rabbits. The findings disclose the limitations of immunization-experiments of the sort outlined as a means of demonstrating "masked" virus. Since much antibody is present in the blood of cottontails having cancers deriving naturally from the virus-induced papillomas, and since extravasated antibody has been demonstrated in extracts of the cancers,[7] it follows that the attempted immunization of normal rabbits with extracts of these growths will fail to provide decisive evidence as to whether the virus is or is not present in the malignant tissue, unless the effects of extravasated antibody can first be excluded.

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6 Kidd, J. G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64**, 63, 79; Kidd, J. G., *J. Exp. Med.*, 1938, **68**, 703, 725, 737.

7 Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1940, **71**, 469.