print more definite information on the subject in the near future, when the university will have more complete records.

The whole story can obviously not be told because so much of the work is of a secret character. But, as Mr. Conant says in his annual report, "if the whole story could be told, it would demonstrate a record of national service among members of the university staff which would be a deep source of pride to all Harvard men." He writes:

> The first asset of a modern university is neither its invested capital nor its plant. The first asset is the faculty. In all our attempts to conserve dollars during the war emergency, therefore, we must be certain that we do not impair the quality of our staff. . . .
>
> During the war years and particularly when hostilities cease, we must bend every effort to strengthen each of our dozen faculties by the addition of the ablest teachers and the most distinguished scholars available. If we succeed in this endeavor we shall insure a brilliant future for this university, whatever fluctuations in our financial fortunes may occur. If we fail, it will be of little moment to the nation whether the figure representing Harvard's dollar assets has moved up or down.—*Harvard Alumni Bulletin.*

SPECIAL ARTICLES

A FACTOR IN DOMESTIC RABBIT PAPILLOMA TISSUE HYDROLYZING THE PAPILLOMA VIRUS PROTEIN[1]

THE virus of infectious papillomatosis[2] is seldom demonstrable[3] in domestic rabbit growths. On the other hand, growths occurring under natural conditions in cottontail rabbits usually yield highly infectious extracts.[2] Further, the virus can be obtained as a homogeneous protein from growths in cottontail rabbits but not from growths in domestic rabbits.[4] The chief reason for suspecting virus in most domestic rabbit warts is the presence of a specific antigen which immunizes[5] other rabbits against infection with the virus. Even in this respect, there is evidence that virus as such is not present in suspensions of the growths, for the antigen is retained by Berkefeld filters, through which the virus readily passes, and is sedimented in ultracentrifugal fields which do not affect the virus. An explanation for the absence of virus and an insight into the probable nature of the antigen of domestic rabbit warts is suggested in the results of the experiments described here.

There is evidence that the papilloma virus is a macromolecular nucleoprotein.[6,7,8] The protein, introduced into susceptible hosts, gains entrance into epidermal cells and there progressively increases in quantity in cottontail and, presumably, also in domestic rabbits. It is conceivable that in these cells there exist not only factors which participate in the synthesis of the virus protein but others which may degrade it. In domestic rabbits the activity of the degrading factors may keep pace with that of factors influencing synthesis. The present work demonstrates a factor, presumably an enzyme, in domestic rabbit wart tissue which hydrolyzes the papilloma virus protein.

TABLE 1

THE EFFECT OF DOMESTIC RABBIT PAPILLOMA TISSUE ON THE PAPILLOMA VIRUS PROTEIN AS MEASURED BY FORMOL TITRATION. THE AMINO NITROGEN WAS DETERMINED BY THE VAN SLYKE METHOD ON THE TRICHLORACETIC ACID FILTRATE

		cc 0.01 N NaOH		
	Time (hours)	Papilloma tissue + virus protein	Papilloma tissue	Virus protein
	0	2.0	2.0	0.43
	3.3	2.4	1.8	0.43
	21.5	3.6	2.4	0.43
	30.5	4.5	3.2	0.78
Total virus protein N	0	0.3 mg		0.3
Amino N found (Van Slyke)	30.5	0.16 mg*		0
Amino N equivalent (from formol titration)		0.13 mg		
Percentage of total N as Amino N (Van Slyke)		53		0

* In excess of the amount present in the filtrate of papilloma tissue alone.

Table 1 shows a typical experiment with papilloma tissue from the domestic rabbit. The rabbit was inoculated broadcast with the virus protein in scarified areas on the abdomen and sides. The resulting confluent growths were sliced off when 1–2 mm high, washed free of blood in 0.9 per cent. sodium chloride solution, and ground with sand in 0.05 M phosphate buffer pH 6.5. The tissue suspension was decanted, diluted and 1.0 cc was added to each of 2 tubes. To one tube 1.0 cc of solution containing 2.0 mg of virus protein was added and to the second 1.0 cc of water. In a third tube 1.0 cc of buffer was added to 1.0 cc of

[1] This work was aided by the Dorothy Beard Research Fund and by a grant from Lederle Laboratories, Inc., Pearl River, N. Y.

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[3] R. E. Shope, *Proc. Soc. Exp. Biol. and Med.*, 32: 830, 1935.

[4] J. W. Beard, W. R. Bryan and R. W. G. Wyckoff, *Jour. Infect. Dis.*, 65: 43, 1939.

[5] R. E. Shope, *Jour. Exp. Med.*, 65: 219, 1937.

[6] D. G. Sharp, A. R. Taylor, D. Beard and J. W. Beard, *Jour. Biol. Chem.*, 142: 193, 1942.

[7] H. Neurath, G. R. Cooper, D. G. Sharp, A. R. Taylor, D. Beard and J. W. Beard, *Jour. Biol. Chem*, 140: 293, 1941.

[8] A. R. Taylor, D. Beard, D. G. Sharp and J. W. Beard, *Jour. Infect. Dis.* Submitted for publication.

virus protein solution. 0.2 cc of 0.1 M NaCN and 0.1 cc of toluene were added to all tubes which were then incubated at 37° C. Immediately and at intervals samples were withdrawn for formol titration. At the end of the experiment an equal volume of 20 per cent. trichloracetic acid was added to the remaining solutions, and the amino nitrogen was determined on the filtrates by the Van Slyke method.

Table 1 shows that the papilloma tissue itself undergoes autolysis, but in the presence of the virus protein more carboxyl groups are liberated. Since the virus alone remains stable, this increase in carboxyl groups may be attributed to the hydrolysis of the virus by the papilloma tissue. The results of the amino nitrogen determination on the trichloracetic acid filtrate confirm this; 53 per cent. of the total virus protein nitrogen was liberated as free amino groups. Under exactly similar conditions the virus was not hydrolyzed by papilloma tissue from cottontail rabbits, although the tissue alone showed some autolysis. The virus was also not hydrolyzed when it was incubated with normal domestic rabbit epidermal cells[9] treated in the same way.

It appears that the papilloma virus protein is hydrolyzed by some factor, presumably an enzyme, in domestic rabbit papilloma tissue which was absent in the cottontail rabbit wart tissue thus far studied. In the experiment shown in Table 1 the weight of papilloma tissue was not more than 0.2 gm, and the quantity of virus changed was 1.05 mg. Other experiments gave similar results, and the rate of hydrolysis was not increased by doubling the virus protein concentration. Crystalline horse serum albumen and the macromolecular component of normal chick embryo[10] were not hydrolyzed by the domestic rabbit papilloma tissue. The indication is that the catalyst is specific for the virus protein or similar compounds.

The results demonstrate a mechanism by which the papilloma virus may be degraded as fast as it is formed in domestic rabbit papilloma tissue. It is possible, though no evidence of it was seen here, that such a mechanism may operate in lesser and varying degree also in cottontail rabbits to account for variations in the virus content, especially in experimentally induced growths.[4] The findings are not incompatible with the antigenicity of domestic rabbit wart material, for the antigen could well be a non-infectious, insoluble and possibly partially denatured degradation product of the virus, somewhat analogous to the degraded virus antigen of equine encephalomyelitis vaccines.[11] The possibility exists that a similar mechanism accounts for the absence of virus from the carcinoma based on the papilloma of domestic rabbits,[12] as well as that which derives from papillomas of cottontail rabbits.[13] The cells of the latter may acquire virus-degrading factors or enzymes in the carcinomatous change. It is an obvious possibility that such factors may prevent the recovery of a causative agent from neoplastic growths other than those associated with the papilloma virus.

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THE RESPIRATION OF ELODEA

MEASUREMENTS of respiration on higher aquatic plants are seldom performed. The present investigation, originally to determine the relation of cation intake to gaseous metabolism, provided a better insight into the metabolic implications of a hydrophytic existence. Slight modifications of the Fenn-Ledebur[1] microrespirometer allowed simultaneous measurement of carbon dioxide production and oxygen consumption of excised leaves of *Elodea canadensis* Michx. with facility. A barium hydroxide solution was used to absorb the carbon dioxide, and its conductance was measured at intervals by the use of the Kohlrausch bridge method. Experiments averaged three hours in length and were performed in the dark.

Respiratory quotients were very high (average 8.4), suggesting the possible occurrence of anaerobiosis, possibly in connection with or in addition to the utilization of chemically bound oxygen. Presence of previously stored substrates rich in chemically bound oxygen would decrease the amount of free oxygen necessary for complete combustion, would lower the apparent oxygen consumption, and thus cause the R.Q. to rise. Substances like oxalates and citrates would serve well as such substrates; in fact, calcium oxalate has been identified in cell vacuoles of elodea leaves.[2] Migration of oxalic, citric or other acids to

[9] For this, layers of cells were thinly shaved from the ears and abdominal skin. The results are of dubious significance for the amount of cells thus obtainable is minute.

[10] A. R. Taylor, D. G. Sharp, D. Beard and J. W. Beard, SCIENCE, 94: 613, 1941.

[11] D. G. Sharp, A. R. Taylor, H. Finkelstein, D. Beard and J. W. Beard, *Proc. Soc. Exp. Biol. and Med.*, 43: 650, 1940.

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[13] J. G. Kidd and P. Rous, *Jour. Exp. Med.*, 71: 469, 1940.

[1] Wallace O. Fenn, *Am. Jour. Physiol.*, 84: 110, 1928. J. Frhr. v. Ledebur, *Mikrochemie*, Pregl-Festschrift (Sonderband), 253, 1929.

[2] Daniel Mazia and Jean M. Clark, *Biol. Bull.*, 71: 306, 1936.