in about one part per 100,000, and to cucumber mosaic virus protein which possibly may occur in less than a part per million. It seems likely that, with respect to concentration in the host and to instability, certain of these viruses are much more nearly comparable to many animal viruses than is the very stable and abundant tobacco mosaic virus. As a whole, the results demonstrate that high molecular weight proteins are characteristic of these various virus diseases, and that the physical, chemical and serological properties and the concentration in the host of these proteins differ widely.

SUMMARY

A high molecular weight crystalline protein, possessing the properties of ring spot virus and differing markedly from tobacco mosaic virus protein in its physical, chemical and serological properties, has been isolated by means of an ultracentrifuge from Turkish tobacco plants diseased with tobacco ring spot virus. Ultracentrifugal methods were also used to demonstrate that high molecular weight proteins are characteristic of other virus diseases. The concentration of the different virus proteins in the host was found to differ greatly. The quantity ultracentrifuge, used in conjunction with an analytical ultracentrifuge, has proven to be a powerful tool for the concentration, purification and crystallization of high molecular weight virus proteins and to be indispensable in the case of unstable viruses existing in low concentration in the host.

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VITAMIN B_1 A GROWTH FACTOR FOR HIGHER PLANTS

IN experiments to be reported in detail elsewhere, we have found that vitamin B_1 is an important "growth factor" or "growth hormone" for growth *in vitro* of isolated roots. It seems probable that vitamin B_1 is the active principle of yeast extract, shown by Robbins[1] to be beneficial for the growth of isolated corn roots, and by White[2] to be necessary for the continued growth of isolated tomato roots.

After an extensive search for optimal conditions and optimal composition of the nutrient solution it was first found possible to grow freshly isolated pea roots in a pure synthetic medium containing inorganic salts and sucrose. Additions of yeast extract had no stimulating effect upon this initial culture or "passage" and, in fact, yeast extract concentrations higher than 0.01 per cent. were slightly inhibitory, due probably to heteroauxin present in the yeast. If such roots were subcultured by the removal of 10 mm tips into fresh medium and particularly if this procedure were repeated several times, yeast extract was, however, found to be essential for growth. Thus in the third passage pea roots, cultivated in nutrient medium but without yeast, ceased growth completely, whereas roots in the same medium but with the addition of 0.01 per cent. yeast extract may be carried through many passages with an average growth rate of 6 to 9 mm per root per day. The pea root as cut from the seedling plant contains thus sufficient "growth factor" to permit of growth for some time and the initial culture is not influenced by yeast extract, since this growth factor is not limiting. After two or more passages this initial supply is, however, used up and the root responds to growth factor present in the yeast.

It was next found that vitamin B concentrates are considerably more active as a source of the root growth factor than is yeast. This suggested that vitamin B_1 itself might be the active principle and experiments carried out with Merck's crystalline preparation have shown that this is the case. Table I shows that 0.2 gamma per cc is able to replace the optimal yeast extract concentration completely and is in fact superior to it.

TABLE I

GROWTH RATE OF EXCISED PEA ROOTS IN MM PER ROOT PER PASSAGE

Passage	I	II	III	IV	V
No addition	65	10	0	0	0
0.01 per cent. Yeast ext.	64	43	45	40	55
Cryst. B_1 0.2 γ/cc	65	72	65	66	65

Much smaller concentrations of crystalline vitamin B_1 than 0.2 gamma per cc suffice. Thus 0.002 gamma per cc still has a marked stimulating effect upon the growth of these roots. Two gamma per cc has on the other hand no more effect than does 0.2 gamma per cc.

We have as yet no indication that substances other than vitamin B_1 (for example, amino acids in small amounts[3]) are necessary as "growth substances" for pea roots. It is possible, however, that over larger numbers of passages such co-growth substances may be indispensable.

Vitamin B_1 is then not only an animal vitamin and a growth substance for fungi and bacteria, but it is also a growth substance for higher plants. Kögl and Haagen-Smit[4] in a paper published while the above

[1] W. J. Robbins, *Bot. Gaz.*, 74, 59, 1922.
[2] P. R. White, *Plant Physiol.*, 9, 585, 1934.
[3] P. R. White. Paper read at the annual meeting of the Amer. Soc. of Plant Physiologists, Atlantic City, December, 1936.
[4] F. Kögl and A. Haagen-Smit, *Zeit. Physiol. Chemie*, 243, 209, 1936.

experiments with crystalline vitamin B_1 were in progress confirm this conclusion, in that they have shown that B_1 is beneficial to the growth *in vitro* of excised pea embryos, the effect being apparently principally upon the root.

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THE SPARING EFFECT OF DOG DISTEMPER ON EXPERIMENTAL POLIOMYELITIS[1]

WE wish to report a disease produced in rhesus monkeys by the virus of dog distemper and the sparing effect it has on subsequently induced poliomyelitis.

Distemper virus from ferret spleen was inoculated into rhesus monkeys intracerebrally, subcutaneously and intraperitoneally as well as by combinations of these methods. From 0.2 to 0.5 cc of the supernatant fluid of a 20 per cent. emulsion of splenic tissue was used. Twenty-eight monkeys have been infected in this fashion and all have contracted a characteristic and uniform disease which strikingly resembles distemper. The incubation has been from 3 to 9 days, the febrile reaction has lasted about three weeks and rhinitis, conjunctivitis, red streaks about the eyes, weakness and slight incoordination have been the usual symptoms. Only one of the animals died of distemper. This monkey developed encephalitis and expired seven weeks after inoculation. Two other animals were successfully infected from an emulsion of his brain and the disease has also been passed from monkey to monkey by injection of infected blood.

Twenty-five of the monkeys suffering from distemper were later given poliomyelitis (0.2 cc of the supernatant fluid of a 10 per cent. cord emulsion). This virus has regularly produced poliomyelitis in our laboratory with a mortality of 100 per cent. However, in the animals suffering from distemper the results were entirely different. The mortality rate was only 33 per cent. and an equal number recovered without residual paralysis. The animals which did die differed from the controls in that paralysis was delayed.

The results are interesting in that they show the protective power of a relatively benign disease on one otherwise invariably fatal, in the degree to which protection has been afforded monkeys against poliomyelitis and because they suggest the existence of a new immunity mechanism in the virus field.

TABLE I
EFFECT OF DISTEMPER ON COURSE AND OUTCOME OF POLIOMYELITIS IN RHESUS MONKEYS

Group	Animal number	Days after distemper inoculation poliomyelitis was given	Incubation of poliomyelitis in days to paralysis	Outcome		Number of extremities paralyzed	
				Recovered	Died		
III	56	4	no paralysis	×		0	
	58	4	13	×		1	
	60	4	13		×	all	
	62	4	13	×		2	
Control	31		7		×	all	
I	42	7	13	×		2	
	43	7	13	×		3	
	38	7	no paralysis	×		0	
	48	7	12		×	3	Polio death on 24th day
Control	49		8		×	all	
IV	57	9	no paralysis	×		0	
	59	9	no paralysis		×	0	Lobar pneumonia 16th day
	61	9	no paralysis	×		0	
	63	9	12		×	4	
Control	74		8		×	all	
V	64	13	15	×		2	
	66	13	no paralysis	×		0	
	68	13	7		×	all	Polio death 7th day
	70	13	no paralysis	×		0	
Control	75		7		×	all	
VI	65	20	11		×	all	Polio death on 11th day
	69	20	12	×		2	
VII	37	70–20	13	×		4	
	39	70–20	8		×	all	
	44	70–20	9	×		4	
Control	53		8		×	all	
II	36	31	6		×	all	Group indistinguishable from control animals
	40	31	6		×	all	
	46	31	6		×	all	
	47	31	6		×	all	
Control	51		5		×	all	

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[1] From the laboratories of Grasslands Hospital, Valhalla, New York.