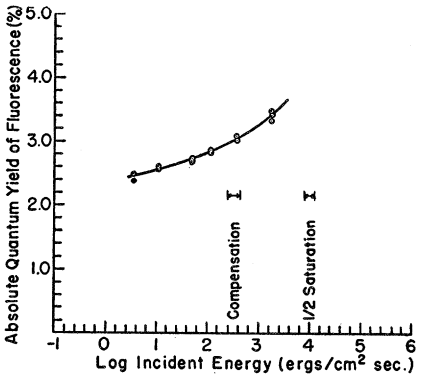

Fig. 1. Quantum yield of fluorescence of *Chlorella* cells. Yield is shown as a function of the logarithm of the intensity of the exciting beam (averaged over its path in the vessel). $\lambda_{ex.} = 436$ mμ. The same values plotted on a linear rather than semilogarithmic scale lead to a curve that is concave downward rather than upward.

photosynthesis-saturating intensities, is not applicable at the low intensities studied here. Apparently, the intensity dependence of ϕ *in vivo* is due to two (or more) factors that come into play in different intensity ranges. The change in ϕ near or below the compensation point may reflect the participation in photosynthesis of respiratory intermediates whose relative importance must decrease as the intensity increases.

We did not observe an inflection in the $\phi = f(I)$ curve corresponding to the one reported by Kok (*8*) for photosynthesis. But kinetic considerations show that, even if different factors govern ϕ primarily in different intensity regions, such inflections would not necessarily occur.

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Sperm Transport in the Reproductive Tract of the Female Rabbit

Previous estimates of the time required for sperm ascent in the reproductive tract of the female rabbit usually have been based on flushing various regions of the tract at definite intervals after mating (*1, 2*). The present note offers another approach to the problem—namely, tubal ligation at various times after mating and the subsequent examination of the trapped eggs for evidence of fertilization. While this work was in progress, a paper using the same technique with several variations appeared (*3*); the results reported here confirm Adams' findings.

Mature New Zealand giant white does were used in the experiments (*4*). The rabbits were bred once to males of proved fertility. At intervals of 0.5, 2, 3, 4, and 5 hours *post coitum*, laparotomies were performed, and the fallopian tubes were doubly ligated and sectioned at the uterotubal junction. The rabbits were killed between 48 and 52 hours *post coitum*, the tubes were flushed with 0.9 percent saline solution, and the recovered eggs were then examined for evidence of normal cleavage and development.

The results indicate that sufficient sperm are in the tubes of every animal by 5 hours *post coitum* to fertilize all viable eggs (Table 1). The increase in the percentage of fertilized eggs between 4 and 5 hours *post coitum* parallels a similar rise in the number of sperm recovered from the tubes during the same time span (*2*). However, it is misleading to account for the increased percentage of fertilized eggs on the basis of an increase in the *mean* number of spermatozoa. The most likely explanation for the increased percentage of fertilized ova is that sperm have reached the tubal level of *every* animal by 5 hours *post coitum*. Before this time, there is considerable individual variation in the rate of sperm entry into the tubes as measured by the number of animals with fertilized ova at different hours *post coitum* (Table 1).

Table 1. Fertilizing ability of rabbit sperm in ligated fallopian tubes. (Eggs examined 48 to 52 hours *post coitum*).

Time *post coitum* tubes ligated (hr)	No. of rabbits	No. of follicular rupture points	No. of eggs recovered	Percentage of fertilized eggs	No. of animals with fertilized ova
½	3	21	20	0	0
2	5	48	31	19	1
3	5	54	53	47	3
4	5	29	25	40	3
5	5	46	41	98	5

Differences from animal to animal in uterine motility and in the mechanical barrier offered by the cervix and uterotubal junction probably account for the variability in the rate of sperm transport before 5 hours *post coitum* (*2, 5*).

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Cold-Adapted Genetic Variants of Polio Viruses

Variants of the polio viruses have been obtained through passage in various *in vitro* systems. Enders, Weller, and Robbins (*1*) passaged the Brunhilde strain (antigenic type I) in tissue cultures of human embryonic skin muscle and obtained a variant of reduced virulence for monkeys. Sabin, Hennessen, and Winsser (*2*) have obtained variants of Mahoney (type I), Y-SK (type II), and Leon (type III), which are also relatively avirulent in monkeys, through passage at 1-day intervals with large inocula in tube cultures of cynomolgus monkey kidney cells. Li, Schaeffer, and Nelson (*3*) have combined passages *in vitro* with passages *in vivo* to obtain variants of Mahoney and Leon which show various patterns of virulence for mice and monkeys. Melnick (*4*) has also reported attenuation of polio viruses through serial passages of high concentrations of virus in tissue culture. Dulbecco and Vogt (*5*) have obtained an *r* (rapid) mutant of Brunhilde through serial rapid passage on monolayer cultures of cynomolgus monkey kidney cells. Slow Mahoney (*6*), a genetic variant of Mahoney that produces relatively tiny plaques on monolayers of monkey kidney cells, was isolated after propagation of the parental Mahoney on HeLa cells. In the work reported here (*7*), cold-adapted genetic variants of the polio virus strains Akron (type I), Brooks (type II), and Mabie (type III) have been obtained through passage at 30°C on monkey kidney cells.

Table 1. Comparisons of original polio viruses and cold-adapted derivatives in time required for completion of cytopathogenic action on monkey kidney cells in stationary tubes at 30° and 36°C.

Polio virus strain and pool	Temperature (°C)	Time required for completion of cytopathogenic action (mean day tubes positive)		
		10,000 PPP*	1000 PPP	100 PPP
Original Akron (PP1)	30	6.0	7.4	≧8.2
Cold-adapted Akron (PP3)	30	3.2	4.0	4.4
Original Akron (PP1)	36	3.4	3.8	4.3
Cold-adapted Akron (PP3)	36	3.4	4.5	4.8
Original Brooks (PP1)	30	4.8	4.8	6.0
Cold-adapted Brooks (PP3)	30	3.4	4.2	4.8
Original Brooks (PP1)	36	3.2	3.2	3.6
Cold-adapted Brooks (PP3)	36	3.2	3.6	3.7
Original Mabie (PP1a)	30	4.7	6.1	7.9
Cold-adapted Mabie (PP3)	30	3.2	3.2	4.5
Original Mabie (PP1a)	36	3.2	3.4	3.7
Cold-adapted Mabie (PP3)	36	6.8	8.4	≧10.0

* Plaque-producing particles (PPP) inoculated per tube. All of the determinations were made on the same lot of monkey kidney cells and each entry is the mean of five or six tubes.

Akron plaque-purified pool No. 1 (PP1), Brooks PP1, and Mabie PP1a (*6*) were selected for passage. The cultures of monkey kidney tissue used for passage were cultures of trypsinized rhesus or cynomolgus monkey kidney cells which had grown for 6 days in tubes at 36°C on medium D (*8*). The cultures were washed once with 0.90 ml of medium 199 (*9*) before inoculation. For each passage, 0.10 ml of virus, usually at 10^{-2} concentration, was inoculated into such cultures containing 0.90 ml of medium 199. The tubes were slanted at 30°C, examined daily for cytopathogenic action, and harvested as soon as at least 90 percent of the cells were destroyed. Ten such serial passages were made with each strain. The tenth passage level of each was then purified through plaque isolation three times serially at 30°C. A pool was formed at 30°C in bottles of monkey kidney cells from the third plaque passage of each.

The 30°C-passaged viruses, in comparison with the original viruses, have more rapid cytopathogenic action on monkey kidney cells in tubes at 30°C (Table 1). Furthermore, these cold-adapted variant particles produce larger plaques at 30°C than do their progenitor particles.

Curves that show the propagation of the original viruses and their cold-adapted derivatives on monkey kidney cells at 30° and 36°C have been determined (Fig. 1). Supernatant fluids of tube cultures were harvested daily and assayed by the plaque method of Dulbecco and Vogt (*10*). These curves show that the cold-adapted variants propagate more rapidly at 30°C than do their progenitors.

Plaques formed by the original viruses at 30°C and by the cold-adapted variants at 36°C have been isolated, and the progeny particles in these plaques have been studied with respect to their cold adaptation. In each case, the nature of the progeny particles was a function of the nature of the parental particle and not a function of the environment—that is, the temperature, in which they were formed. Thus these cold-adapted pools are composed, at least predominantly, of polio virus particles that are genetically altered in their capacity to propagate on monkey kidney cells at 30°C.

Whereas all three cold-adapted variants are partially deadapted to propagation at 36°C in monkey kidney tissue cultures (see Table 1 and Fig. 1), the deadaptation of Mabie is much greater than that of Akron or of Brooks. It is possible that these cold-adapted variants may be generally deadapted to propagation at temperatures as high as 36°C and, hence, may possess less virulence for a primate whose body temperature is normally somewhat greater than 36°C. Therefore, these cold-adapted variants have been compared with the original viruses in virulence for rhesus and cynomolgus monkeys by the intraspinal route of inoculation (*11*). The data available at this time show that the cold-adapted variants of Akron and of Brooks do not differ in virulence from their progenitors. The cold-adapted Mabie, however, has been found to be less virulent (*12*) than the original Mabie. Thus, the strain that has been strikingly deadapted to propagation at 36°C in tissue culture is also the one that has become less virulent for the monkey. This result suggests that the degree of loss of ability of 30°C-passaged viruses to propagate at 36°C in tissue cultures may serve as an indicator of the degree of their loss of virulence for a warm-blooded animal.

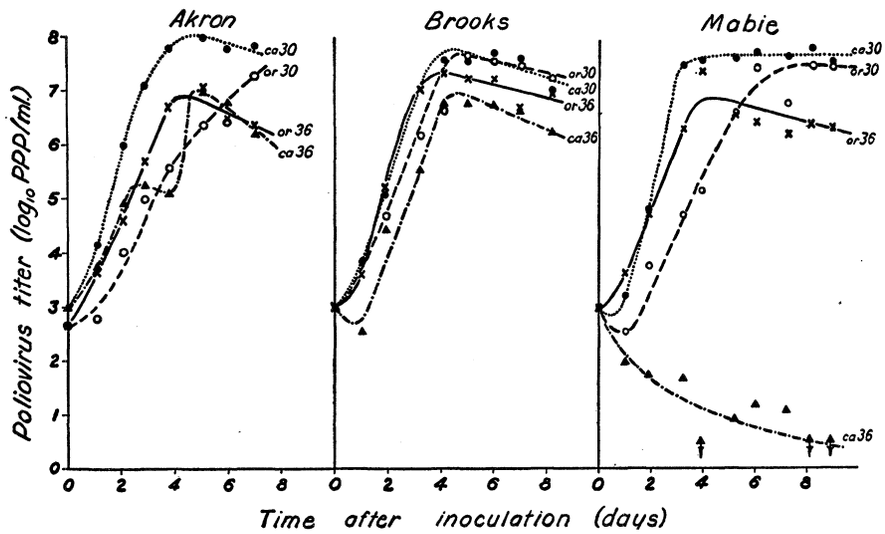

Fig. 1. Propagation curves of original and cold-adapted polio viruses on monkey kidney cells at 30° and 36°C. Original viruses, *or*; cold-adapted derivatives, *ca*. The temperature at which viruses were grown follows the symbol. The arrows indicate that the values recorded are maximal; that is, no virus was found in the aliquots assayed. All harvests of any one of the three strains were made from the same preparation of monkey kidney cells and consisted usually of the pooled fluids from two tube cultures selected at random from many replicate inoculated cultures.

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Influence of Crystalline Elastase on Experimental Atherosclerosis in the Chicken

Elastase is a pancreatic enzyme that has been studied and described by Baló and Banga (*1*). Lansing (*2*) and Carter (*3*) have determined that it comes from islet tissue and specifically from the α-cells. Preparation of crystalline elastase from beef pancreas has been reported by Banga (*4*) and from pork pancreas by Lewis, Williams, and Brink (*5*).

It is thought that there may be a connection between elastase and arteriosclerosis. Baló and Banga (*6*) noted that men suffering from arteriosclerosis had less elastase in the pancreas than did healthy individuals, and Lansing (*7*) gave elastase by mouth to cholesterol-fed rabbits and found that it retarded the development of atheromatosis.

We report here the influence of crystalline porcine elastase (*8*), given orally and parenterally, and of trypsin, included as a control for the proteolytic action, on atheromatosis and plasma lipid pattern in cholesterol-fed chickens. Two preparations of crystalline material containing 130 and 134 elastase units per milligram were tested in separate experiments 24 weeks apart. The procedures are described in more detail elsewhere (*9*).

For each test, six groups of 8-week-old White Leghorn cockerels, raised on starter ration, were set out on diet containing 2 percent USP cholesterol and 5 percent cottonseed oil and treated as follows: (i) no further treatment; (ii) diet fortified with 57 mg of crystallized trypsin (Worthington) per kilogram; (iii) diet fortified with 57 mg of elastase per kilogram; (iv) given 0.2 ml of saline intramuscularly five times per week; (v) given 4 mg of crystallized trypsin intramuscularly five times per week; (vi) given 4 mg of elastase intramuscularly five times per week.

After 8 weeks of treatment, the birds were fasted overnight, bled, and sacrificed. Four milliliters of blood was drawn from the alar vein of each bird and mixed with 0.7 ml of solution of citric acid, sodium citrate, and dextrose (ACD solution) (*10*). The prepared plasma samples were analyzed for total cholesterol (*11*) and lipid phosphorus (*12*) and for cholesterol in α- and β-lipoprotein after fractionation by Cohn's method 10 (*10, 13, 14*). The thoracic aortas and brachiocephalic arteries were removed and examined for degree of atheromatosis by two independent observers. A score of 1 was assigned for thin, scattered plaques; a score of 2 for either light, uniform deposit or heavy, scattered plaques; a score of 3 for heavy, uniform deposit; and a score of 4 for extremely heavy and lumpy deposit.

The results are shown in Table 1. Lipid concentrations in ACD plasma should be multiplied by 1.3 to obtain corresponding serum values.

Food consumption data showed that the average enzyme intakes by birds on the dietary regimens in the first experiment were 4.9 mg of trypsin per day and 4.4 mg of elastase per day. In the second experiment, the intakes were 4.5 mg of trypsin per day and 4.3 mg of elastase per day. Samples of different lots of diet were analyzed by U. J. Lewis, who found that there was no loss of enzyme activity in them before they were consumed.

The two experiments differed in the severity and incidence of atheromatosis. The injected birds seemed to have higher α-lipoprotein cholesterol and a higher $\alpha/(\alpha+\beta)$ ratio than those on dietary regimens.

Crystalline elastase, given either in the diet or by intramuscular injection, did not reduce either incidence or severity of atheromatosis in cholesterol-fed chickens. The elastase-treated birds gained less weight on the average, and had slightly more α-lipoprotein cholesterol ($p < 0.05$) than their companions. The lipid patterns were not otherwise influenced in a direction that would be considered beneficial in man.

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Table 1. Lesion scores, plasma cholesterol concentrations, and distributions between lipoproteins, plasma lipid-phosphorus concentrations, ratios of cholesterol to phospholipid (C/PL), and weight changes in control and treated cockerels.

Substance	Lesions		Cholesterol (mg/ml)				Lipid P (μg/ml)	C/PL	Wt. gain (g)
	Incidence	Avg. score	Total	α-Lipoprotein	β-Lipoprotein	$\frac{\alpha}{\alpha+\beta}$			
Experiment 1, Enzyme in the diet									
None	5/10	0.9	3.56	0.27	3.29	0.12	55.0	2.21	939
Trypsin	4/10	0.6	2.32	0.26	2.02	0.13	40.4	2.27	1042
Elastase	8/10	1.55	4.40	0.37	3.89	0.11	55.4	3.00	927
Experiment 1, Intramuscular injection									
Saline	4/10	0.45	2.14	0.33	1.83	0.16	43.4	1.94	984
Trypsin	3/7	1.0	3.33	0.35	2.78	0.14	54.7	2.37	896
Elastase	3/10	0.5	4.28	0.65	3.34	0.16	63.3	2.50	775
Experiment 2, Enzyme in the diet									
None	7/10	1.7	4.75	0.37	4.39	0.10	60.3	2.99	1007
Trypsin	8/10	1.5	3.31	0.33	2.98	0.11	54.7	2.37	1007
Elastase	9/10	1.95	4.54	0.39	4.16	0.09	62.3	2.85	966
Experiment 2, Intramuscular injection									
Saline	8/10	1.7	4.68	0.41	4.17	0.12	63.6	2.99	957
Trypsin	7/9	1.45	4.41	0.46	3.88	0.13	64.5	2.51	918
Elastase	8/9	1.9	4.66	0.52	4.09	0.12	64.6	2.66	891

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