The samples of infected cells from all these stages were also stained with anti-SPV antiserum. No significant amount of SPV-antigen was detectable in cells of any of the samples examined.

Discussion and Conclusion. Embryonic skin culture of domestic rabbit was infected with Shope papilloma virus *in vitro* and the early phase of virus-cell interaction was studied sequentially up to 30-hours post infection by the immunofluorescence technique. The cells of infected cultures showed a specific fluorescent reaction in the nucleus when stained with antipapilloma antisera conjugated with FITC. The ratio of positively stained cells was about 20% of the whole cell population in cultures 5–6 hours after infection with the virus and it reached a maximum of approximately 90% at around 11 hours. The morphological pattern of the intranuclear fluorescent staining was reminiscent of the reaction of T antigen reported in the SV40-infected hamster cells (6).

Kreider *et al.* (7) have recently reported their findings on the persistence of Shope papilloma virus absorbed onto the cell surface of cells of *in vitro* cultured embryonic skin of rabbits. The viral antigen was detectable in cultured cells even after 6-days post-infection by immunofluorescence. The fluorescent granules were seen randomly distributed all over the cell in most of the cases.

The specific immunofluorescence in the SPV-infected embryonic cells as shown in the present study was clearly associated with the nucleus and the positive staining reaction was only induced by the antipapilloma antiserum. Experiments with the anti-SPV antiserum yielded only negative results. From these findings, it seems plausible to conclude that the antigen demonstrated in the nucleus of the SPV-infected rabbit cells by immunofluorescence possibly meets at least a part of the criteria of T antigen. Studies are being carried out to assess the problem from different approaches such as complement fixation test between the cellular extract of the infected cell culture and the antipapilloma and anti-SPV antisera.

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Specificity in Protection against Lethality and Testicular Toxicity from Cadmium* (33074)

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Cadmium salts, administered subcutaneously, cause selective destruction of the testis (1–3). Selenium compounds are very effective in blocking this reaction (4,5) and we questioned whether they might also diminish the generalized acute toxicity of larger amounts of cadmium. The following experiments were undertaken in male mice to determine if doses of cadmium, normally fatal,

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could be administered safely in the presence of selenium. Comparisons were also made on the effect of selenium on the lethal potency of two elements related to cadmium, zinc and mercury, as well as on another metal, cobalt. Since 2,3-dimercaptopropanol (BAL) and cysteine (6), as well as salts of zinc (1, 3–5) and cobalt (7), also prevent testicular damage from cadmium, these compounds were investigated too for their possible influence on the lethality of cadmium.

Materials and Methods. Male albino mice (CD-1 strain from The Charles River Mouse Farms, Inc.) 20 weeks of age, weighing 40 gm, were used in these experiments. From 4–6 mice were used for each dose tested. For studies of testicular damage with sublethal doses of cadmium, mice were killed 6 days following injection and the testes were removed, fixed in Bouin's fluid and processed by routine histological techniques for subsequent microscopic examination. For studies with lethal doses of chemicals daily checks were made for mortality. The mice which did not die from the high doses were killed and the testes were taken for microscopic study.

Toxic agents. Cadmium. To test the capacity of cadmium to cause testicular necrosis, mice received 0.008–0.020 mmole/kg of cadmium chloride (Cd) administered as a single 0.2-ml subcutaneous (s.c.) injection of 0.0016–0.0040 *M* aqueous (aq) solution. For lethality studies, mice received larger doses of Cd, 0.038–0.133 mmole/kg, administered as a single 0.2-ml s.c. injection of 0.0760–0.0266 *M* aq solution.

Mercury. As a lethal dose, mice received 0.09 mmole/kg of mercuric chloride (Hg) administered as a single 0.2-ml s.c. injection of 0.018 *M* aq solution.

Zinc. For studies on lethality, 6 mmole/kg of zinc acetate (Zn) were administered. Mice received two 0.4-ml injections of 0.3 *M* aq solution simultaneously at different s.c. sites.

Cobalt. As a lethal dose, mice received 1.5 mmole/kg of cobaltous acetate (Co), administered as a single 0.2-ml s.c. injection of 0.3 *M* aq solution.

Protective agents. Selenium. For the various doses used, 0.006–0.096 mmole/kg, mice received a single s.c. injection of 0.0012–0.0192 *M* aq solution of selenium dioxide (Se). When administered with the toxic agents, Cd, Hg, Zn, or Co, it was given at the same time but at a different s.c. site.

TABLE I. Minimal Doses of Se, Zn, Co, BAL, and Cysteine Needed to Block Testicular Injury Produced by Cd (0.012 mmole/kg).

Protective agent	Dose (mmole/kg)	Ratio (agent:Cd)
Se	0.024	2:1
Zn	0.15	>12:1
Co[a]	0.108	9:1
BAL	0.75	>62:1
Cysteine	6.0	500:1

[a] More recent studies indicate this dose may be lowered to 0.072 mmole/kg or below (ratio of 6:1 or less) if Co is administered 24 hours prior to Cd.

Zinc. As a protective agent, Zn was administered in doses of 0.1–0.5 mmole/kg. Mice received a single 0.2-ml s.c. injection of 0.02–0.10 *M* aq solution. When administered with Cd it was given 24 hours previously and at a different s.c. site.

Cobalt. As a protective agent, Co was administered in doses of 0.072–0.246 mmole/kg as a single 0.2-ml s.c. injection of 0.0144–0.0492 *M* aq solution. When administered with Cd it was given at the same time but at a different s.c. site.

BAL. This compound was administered in doses of 0.50–0.75 mmole/kg as a single s.c. injection of 0.10–0.15 *M* solution in peanut oil. When administered with Cd it was given at the same time but at a different s.c. site.

Cysteine. This amino acid was given in a dose of 6 mmole/kg. Mice received a single 0.8-ml intraperitoneal injection of 0.3 *M* cysteine hydrochloride in the form of a neutralized aq solution. When administered with Cd it was given simultaneously.

Results. Minimal levels of Se, Zn, Co, BAL and cysteine needed for protection against testicular injury by Cd. In preliminary studies it was found that the lowest dose of Cd which produced testicular injury was 0.012 mmole/kg. The minimal amounts of protective agents needed to block this effect consistently are listed in Table I. The subsequent experiments with larger doses of Cd

showed that the testes were not protected unless this ratio of protective salt:Cd was maintained. This study showed considerably more Se (four times) and less Zn (one fourth) was needed for protection of the testes against Cd in CD-1 mice than in Sprague-Dawley rats (5).

TABLE II. Protective Effect of Se Against Cd Lethality in CD-1 Male Mice.

Dose of Cd (mmole/kg)	No. of mice dead by 12 days (5 injected/dose); dose of Se (mmole/kg)[a]			
	0	0.024	0.072	0.096
0.038	0	0	—	—
0.044	1	0	—	—
0.050	2	0	—	—
0.058	5	0	—	—
0.066	4	1	—	—
0.076	3	3	1	—
0.086	5	5	2	—
0.101	5	5	0	2
0.116	5	5	1	—
0.133	5	5	2	5

[a] No deaths from 0.024–0.072 mmole/kg of Se alone; 0.096 mmole/kg was 100% lethal. Testes were not protected from injury by cadmium with either of these doses of Se.

Effect of Se against Cd lethality. Table II indicates that the dose of Se (0.024 mmole/kg) which protected the testis from the minimal dose of Cd (0.012 mmole/kg) also prevented mortality from larger doses of Cd (0.044–0.066 mmole /kg). Lower doses of Se (0.006–0.012 mmole/kg) were inadequate (not shown). Following administration of higher doses of Cd (0.076–0.133 mmole/kg), Se had to be increased to 0.072 mmole/kg before protection against lethality became evident. A dose of 0.096 mmole/kg of Se, in itself 100% lethal, was not as effective against Cd lethality as the dose of 0.072 mmole/kg. Table III shows that Se protection against Cd lethality is still very effective as long as 79 days following injection.

Comparison of effect of Se against lethal doses of Cd, Hg, Zn, and Co. Table IV shows that lethality from Hg, Zn, and Co, in contrast to that of Cd, was neither prevented or delayed by simultaneous administration of

TABLE III. Duration of Se Protection Against Cd Lethality in CD-1 Male Mice.

Salts administered	No. of mice dead (25 injected); no. days following injection				
	3	7	12	26	79
Cd[a]	23	—	—	—	—
Cd + Se[b]	5	—	1	1	2

[a] Represents the 25 mice of Table II which received Cd (0.076–0.133 mmole/kg).

[b] Represents the 25 mice of Table II which received Se (0.072 mmole/kg) and Cd (0.076–0.133 mmole/kg).

Se; indeed the onset of mortality was earlier (particularly in the case of Hg) when Se was also administered.

Comparison of effect of Zn, Co, BAL, and cysteine on Cd toxicity. Table V demonstrates that doses of Zn and BAL which blocked testicular injury from the low dose of Cd were also effective in preventing mortality from larger doses (0.101–0.133 mmole/kg). In contrast, Co and cysteine were ineffective in antagonizing lethality from Cd. Cysteine in fact caused a marked enhancement of Cd lethality.

Discussion. These experiments demonstrated that small amounts of selenium can pro-

TABLE IV. Comparative Effect of Se Against Lethal Doses of Cd, Hg, Zn, and Co in CD-1 Male Mice.

Salts administered[a]	No. of mice dead (6 injected); time following injection					
	(hours)		(days)			
	6	24	2	3	4	7
Cd	0	4	2	—	—	—
Hg	0	0	0	3	2	1
Zn	2	3	1	—	—	—
Co	1	5	—	—	—	—
Cd + Se	0	0	0	0	0	0
Hg + Se	6	—	—	—	—	—
Zn + Se	6	—	—	—	—	—
Co + Se	6	—	—	—	—	—

[a] Doses: Cd and Hg (each 0.09 mmole/kg); Zn (6 mmole/kg); Co (1.5 mmole/kg); Se (0.072 mmole/kg). Only Cd produced testicular injury.

TABLE V. Comparative Effect of Zn, Co, BAL, and Cysteine on Cd Toxicity in CD-1 Male Mice.

Agent administered	Dose (mmole/kg)[a]	No. of mice dead by 12 days/total injected; dose of Cd (mmole/kg)		
		0.026	0.101	0.133
Control	—	0/6	5/5	5/5
Zn	0.15	0/6	2/5	0/5
Zn	0.50[b]	0/6	0/4	1/4
Co	0.108	0/6	5/5	4/5
Co	0.246[b]	0/6	5/5	5/5
BAL	0.75	0/6	0/5	1/5
Cysteine	6.0	6/6	5/5	—

[a] No deaths from any of these agents alone nor did any produce testicular injury.

[b] These doses of protective agent blocked testicular injury from the lower dose of cadmium only (0.026 mmole/kg).

tect against lethality from subcutaneously administered cadmium, bearing out a very early report (8) of the therapeutic effect of this metalloid against fatal doses of inhaled cadmium. Selenium prevents lethality and testicular injury from cadmium even though it causes a marked increase in cadmium levels in blood (9) and testis (9–11). However, the fact that cadmium augments levels of selenium in blood (10, 12) and testis (10, 11) and also diminishes its excretion (12), suggests some sort of binding between the two elements in which cadmium is rendered innocuous (10, 11).

Two other agents which protect the testis against cadmium injury also prevent lethality from cadmium, BAL, as previously known (8), and zinc. All three agents were more efficient in antagonizing the lethal effects of cadmium than they were in protecting the testis. Selenium and zinc in doses approximately equimolar to cadmium prevented mortality, but ratios of selenium:cadmium of 2:1 and zinc:cadmium of 12.5:1 were needed to block testicular injury. Lethality was prevented by ratios of BAL:cadmium of 6:1 whereas approximately 62 times as much BAL as cadmium was needed to protect the testis from damage.

There were certain specific limitations in the interrelationships between the protective agents. Although selenium protected against lethality from cadmium, it did not prevent mortality from other divalent metals, such as the closely related elements, mercury and zinc, or from cobalt. Cobalt blocks cadmium-induced testicular injury, as well as the hemorrhagic lesions seen in sensory ganglia from relatively large doses of cadmium (7), yet it has no protective effect against lethality from cadmium. Cysteine, like BAL, protects the testis from cadmium damage (6); unlike BAL, it does not prevent lethality from cadmium. Doses of cadmium one-fifth of the LD_{50}, when administered with cysteine are fatal by causing acute devastation of renal proximal convoluted tubules (9). Just as these various agents differ in their mechanisms of protecting the testis from cadmium (10, 11), so have they divergent effects on whole body toxicity from cadmium.

Summary. Cadmium, administered subcutaneously to adult CD-1 mice, causes acute destruction of the testis in doses (0.012 mmole/kg) which cause no apparent damage to other organs. This effect can be prevented by administration of sufficient selenium (0.024 mmole/kg), zinc (0.15 mmole/kg), cobalt (0.108 mmole/kg), BAL (0.75 mmole/kg), or cysteine (6 mmole/kg). Selenium (0.072 mmole/kg) also blocks the lethal effects of much larger doses of cadmium (0.076–0.133 mmole/kg but does not protect against mortality from mercury (0.09 mmole/kg), zinc (6 mmole/kg) or cobalt 1.5 mmole/kg). Not all agents which block cadmium-induced injury to the testis are effective in preventing mortality from cadmium. Whereas zinc and BAL (in the protective doses cited above) are good antagonists against lethal doses of cadmium, cobalt is not and cysteine markedly enhances the toxicity of cadmium. Considering the ratio of protective agent to cadmium which is needed, selenium, zinc, and BAL are relatively more efficient in protecting against lethality than in preventing testicular injury from cadmium. These studies illustrate that the organ most vulnerable to injury from cadmium, the testis, is the most resistant to protection.

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The Utilization of Glucose by Normal Glucose-6-phosphate Dehydrogenase and by Glucose-6-phosphate Dehydrogenase Mediterranean* (33075)

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Deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) is a widespread disorder. Efforts to explain its high prevalence in some populations have centered about possible benefits arising as a consequence of absence of this enzymatic activity. The "malaria hypothesis" (1) has received the greatest attention, and although there is a body of evidence suggesting that deficiency of the enzyme may confer some degree of immunity to malaria, this is by no means generally accepted to be the only important factor in maintaining high gene frequencies in all populations. A quite different possibility therefore deserves consideration. It may be that the loss of catalytic activity with regards to glucose-6-phosphate is merely an incidental side effect of a mutation which gives the enzyme molecule some new, beneficial substrate specificity. Indeed, the abnormal glucose-6-phosphate dehydrogenase produced in some common mutations such as G-6-PD Mediterranean uses 2-deoxyglucose-6-phosphate and galactose-6-phosphate more rapidly than does the normal enzyme (2).

Normal glucose-6-phosphate dehydrogenase has been shown to utilize unphosphorylated glucose as a substrate, although high glucose concentrations were required, and the rate of the reaction was extremely slow (3). In the present study, the capacity of one type of mutant G-6-PD, G-6-PD Mediterranean, to utilize this substrate has been investigated. At the same time, some of the kinetic characteristics of glucose utilization by the normal enzyme are described for the first time.

Materials and Methods. Partial purification of glucose-6-phosphate dehydrogenase was carried out as described previously (4). Enzyme preparations purified in this manner are free of 6-phosphogluconic dehydrogenase activity. All enzymatic assays were performed as described previously (4) except that all measurements were carried out at 37°C. Solutions of nonphosphorylated sugars were prepared at least 24 hours before use to assure complete mutorotation.

Results. A. The utilization of unphosphorylated sugars by normal and Mediterranean G-6-PD. Table I presents the utiliza-

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