TABLE 1

ACID FORMATION BY "FOLIC ACID" REQUIRING LACTOBACILLI IN VARIOUS GROWTH FACTOR MEDIA

Organism	Addenda to 10 cc of basal medium*			
	Nil	SLR factor 0.3γ units†	"Folic acid" 0.003γ units†	1 cc culture fluid of *S. lactis* R grown with 0.3γ units of SLR factor per 10 cc of medium
	cc. N/10 acid formed in 3 days at 37°			
Lactobacillus casei	1.1	0.8	6.5	7.0
Lactobacillus delbrückii LD5	1.9	1.6	7.7	8.7
Lactobacillus casei 19	1.0	0.9	2.5	2.4
Lactobacillus bulgaricus 05	1.8	1.4	7.4	8.5

* Essentially as described by Landy and Dicken, *Jour. Lab. Clin. Med.*, 27: 1086, 1942.

† Using *S. lactis* R, the activity of the SLR factor was standardized against a folic acid concentrate. One "microgram unit" is equivalent to one microgram of folic acid of "potency 40,000." For definition of the latter see H. K. Mitchell and E. R. Snell, *University of Texas Publication* No. 4137, 36, 1941. One microgram of factor SLR is equivalent to approximately 5γ units of folic acid.

acid can be readily produced by adding washed cells of *S. lactis* to a water solution of the SLR factor and incubating the mixture for 3 to 4 hours at 30° C. Under such conditions cell growth is largely eliminated.

A survey of factor SLR and folic acid requirements of a number of lactic acid bacteria revealed that *Streptococcus fecalis* 732, *Streptococcus fecalis* F24, *Streptococcus zymogenes* 5C1 and *Streptococcus durans* 98A, also, can develop with either the SLR factor or folic acid (Table 2). Growth of these strep-

TABLE 2

STREPTOCOCCUS LACTIS R FACTOR AND FOLIC ACID REQUIREMENTS OF LACTIC ACID BACTERIA

Organisms requiring		
Factor SLR or folic acid (interchangeable)	Folic acid	Nil
Streptococcus lactis R	*Lactobacillus casei*	*Lactobacillus arabinosus* 17–5
Streptococcus fecalis 732*	*Lactobacillus delbrückii* LD5	*Leuconostoc mesenteroides* 6205†
Streptococcus fecalis F24*	*Lactobacillus bulgaricus* 05	*Streptococcus lactis* 374† [illegible] L103* L104* L206*
Streptococcus zymogenes 5C1*	*Lactobacillus casei* 19	*Streptococcus fecalis* 10C1
Streptococcus durans 98A*	*Streptococcus fecalis* S108A*	*Streptococcus zymogenes* 6054†

* We are greatly indebted to Dr. J. M. Sherman of Cornell University for these cultures. Their folic acid requirements have been determined by Dr. Sherman (personal communication) and our results confirm those obtained by him.

† Obtained from the American Type Culture Collection.

tococci in SLR factor medium is accompanied, in every case, by formation of folic acid. Some lactic acid bacteria, primarily lactobacilli, can not utilize the SLR factor and must be supplied with folic acid for growth. However, the majority of the strains examined do not require either factor for growth, presumably because they are able to synthesize folic acid. Synthesis of folic acid was established for *Lactobacillus arabinosus, Leuconostoc mesenteroides* and *Streptococcus fecalis* 10C1.[3]

As indicated in Table 2, folic acid can replace the SLR factor for all bacteria which can utilize the latter. Also, in every instance folic acid is formed when such organisms are grown with the SLR factor. It, therefore, seems probable that the latter is biologically active because it can be converted to folic acid. If this is the case, the rate of conversion of factor SLR to folic acid must be very rapid, since the rate of growth of *S. lactis* R in media containing these factors is essentially the same[4] (Fig. 1).

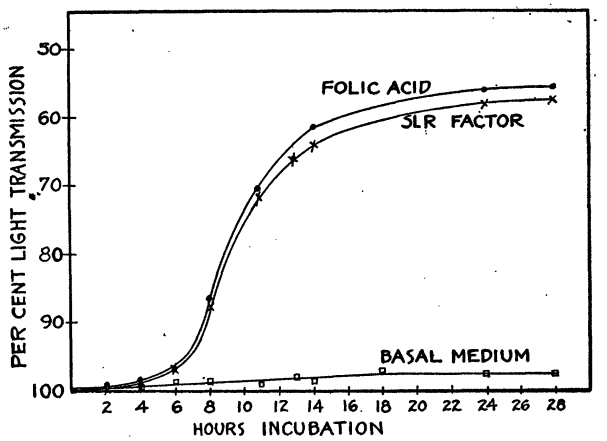

FIG. 1. Rate of growth of *Streptococcus lactis* R in SLR factor and "folic acid" media.

The *S. lactis* R factor, unlike xanthopterin, does not give rise to folic acid when incubated with rat liver suspensions.[5,6]

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THE EFFECT OF ATROPINE SULFATE ON THE COURSE OF INFLUENZA VIRUS INFECTION[1]

IN an investigation of the influence of various factors on the resistance of experimental animals to in-

[3] *Cf.* B. L. Hutchings, N. Bohonos and W. H. Peterson, *Jour. Biol. Chem.*, 141: 521, 1941.

[4] 0.015γ units of each growth factor per 10 cc of medium was used. The cultures were incubated at 30° and growth was measured in an Evelyn photoelectric colorimeter.

[5] L. D. Wright and A. D. Welch, SCIENCE, 98: 179, 1943. An increase in folic acid was obtained with xanthopterin which confirms the results of Wright and Welch.

[6] We are indebted to Miss Marion Gunness and Mrs. Alma Larsen for capable assistance, and Messrs. E. L. Rickes and L. Chaiet for the preparation of the *Streptococcus lactis* R factor.

[1] This work was aided by a grant from the Kresge Foundation.

fluenza A virus infection, it was observed that the administration of a solution of atropine sulfate exerted a marked effect on the course of such infections.

METHOD

The PR–8 strain of influenza A virus[2] was used in these experiments, and mice were inoculated under light ether anesthesia by the intranasal instillation of 0.05 ml of a 1:100,000 dilution of a suspension of infected mouse lung. This inoculum contained approximately 1 m.l.d. of virus. Mice treated with atropine were given 1.0 mg of the drug intraperitoneally. On the basis of the relative toxic dose for man[3] and mice,[4] it was estimated that this amount was approximately 30 times the calculated therapeutic dose for the mouse and about one sixth of the toxic dose. The atropine was administered at various intervals both before and after intranasal instillation of virus under ether anesthesia. All surviving mice were sacrificed after 10 days.

RESULTS

The results of the administration of atropine may be seen in the accompanying table. When the drug was given twelve hours before the virus inoculum, no effect could be detected. If it were given between 15 minutes and 6 hours before the virus instillation, fewer of the atropine treated animals died, the mean survival time of those which did succumb to the infection was greater, and the incidence and extent of the lesions in the treated animals were less than in the controls. The longer the interval between the administration of atropine and virus inoculation, the less marked was this effect. When the atropine was given twelve hours before the virus instillation, the effect could no longer be observed.

Further, atropine administered even as soon as 5 minutes after ether anesthesia and intranasal instillation of virus apparently did not influence the course of the infection. The mortality and extent of the lesions were approximately the same in the treated and untreated mice.

DISCUSSION

Several possible explanations of the ability of atropine to increase resistance to influenza virus infection might be postulated. It has been shown that the resistance of hamsters to influenza virus may be decreased by the intra-tracheal inoculation of virus suspended in gastric mucin[5] or mucus secretions.[6] If one assumes that aspiration of mucus secretions, present in excess following ether anesthesia, may aid in the establishment of the virus infection, then if this excessive secretion were inhibited by the action of atropine, infection would be less likely to occur. Although experiments dealing with the effect of mucin on influenza virus infections were not performed with mice, because of the small size of the trachea, it is believed that susceptibility to influenza virus is greater following ether anesthesia, which is associated with increased mucus secretions of the irritated respiratory tract.

TABLE 1

EFFECT OF INTRAPERITONEAL ADMINISTRATION OF 0.1 ML OF 1 PER CENT. ATROPINE SULFATE SOLUTION AT VARIOUS TIME INTERVALS ON RESISTANCE OF MICE TO INFLUENZA A VIRUS

Time of Administration in relation to virus inoculation.	Number inoculated with virus	Per cent. of deaths	Average time of death in days	Per cent. of animals with pulmonary consolidation among surviving animals		
				100–50 per cent.	49–1 per cent.	0
15 min. before	71	22	7.7	28	18	31
No atropine	63	58	5.8	29	11	3
1 hr. before	29	24	6.8	27	24	24
No atropine	25	44	5.5	40	12	4
3 hrs. before	19	21	8.0	26	32	21
No atropine	17	41	7.0	41	12	6
6 hrs. before	17	47	6.8	24	12	18
No atropine	14	50	7.0	28	14	7
12 hrs. before	17	70	5.5	12	12	6
No atropine	13	61	5.5	23	16	0
5 min. after	44	57	7.0	35	7	2
No atropine	48	52	6.9	31	15	2
10–20 min. after	31	54	7.2	35	10	0
No atropine	30	36	7.5	43	20	0

The administration of atropine after ether anesthesia and virus inoculation did not affect the course of the infection. This is in accord with earlier work from this laboratory,[7] in which it was found that atropine had no effect on the course of experimental pneumococcus pneumonia when the drug was given after the onset of the disease.

SUMMARY

It has been shown in mice that the administration of atropine sulfate intraperitoneally before the intranasal instillation of influenza A virus decreased the incidence and extent of infection of these animals.

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[2] Obtained through the courtesy of Dr. Thomas Francis, Jr.

[3] T. Sollmann, "A Manual of Pharmacology," 5th Edition. Philadelphia: W. B. Saunders Company, 1936.

[4] M. A. Wilberg, *Bioch. Zs.*, 66: 389, 1914.

[5] A. H. Wheeler and W. J. Nungester, SCIENCE, 96:92, 1942.

[6] Unpublished work of the authors.

[7] W. J. Nungester and A. H. Kempf, *Jour. Infect. Dis.*, 64: 288, 1939.