acid metabolism. Since the virus in a tissue-free medium is not affected by DAP, the action is not a direct one. The data presented suggest that DAP interferes with some metabolic process in tissue related to nucleic acid synthesis, and one that is necessary for virus multiplication.

Biesele(16) has reported that DAP, in the concentration used in these studies, preferentially injures Sarcoma 180, as compared to mouse embryonic skin, in a roller tube tissue culture. Hence, it is necessary to exclude the possibility that the results presented here were due to death of the tumor cells caused by DAP and consequent removal of any medium capable of permitting viral multiplication. Four facts provide evidence against this possibility. (1) Although on bioassay most of the tumor is killed in the virus-infected control flasks without DAP, nevertheless the virus can be recovered in high titer. Hence very slight tissue growth permits viral multiplication. Extensive tissue growth in the DAP-treated flasks yielded no virus. (2) Such virus as may survive 48 hours of contact with DAP has lost its capacity to multiply when put into fresh media containing actively growing tissue. (3) Although the Carcinoma 1025 is relatively resistant to the action of DAP, inhibition of viral multiplication also occurs in this tissue. (4) DAP inhibits RSSE *in vivo*(17).

Summary and conclusions. (1) The multiplication of the virus of Russian Spring Summer Encephalitis in tissue culture is inhibited by 2,6-diaminopurine. (2) The action can be blocked as well as reversed by the addition of equimolar concentration of adenine. Guanine is not effective.

16. Biesele, J. J., Berger, R. E., Wilson, A. T., Hitchings, G. H., and Elion, G. B., *Cancer,* 1951, v4, 186.

17. Moore, A. E., and Friend, C., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 153.

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Effect of 2,6-diaminopurine on the Course of Russian Spring-Summer Encephalitis Infection in the Mouse.* (19005)

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The compound 2,6-diaminopurine (DAP) has been shown(1) to be concerned in the synthesis of polynucleotide guanine. It also has been proved to have certain types of biological activity. It prolongs survival time of mice with transplantable leukemia(2). It is seriously injurious to the cells of Sarcoma 180 in tissue culture(3) in concentrations which have no effect on normal embryonic cells growing at the same rate although it exerts no demonstrable effect on the same tumor *in vivo*(4). It has also been shown to have an injurious action on the hematopoietic system of dogs(5) and swine(6) and chick embryo(7). It is known to inhibit the multiplication of vaccinia virus *in vitro*(8) while having no effect *in vivo*. The demonstration that it inhibits multiplication of Russian Spring Summer Encephalitis virus (RSSE) in

* This work was supported by funds from the National Cancer Institute of the U. S. Public Health Service, American Cancer Society and the Damon Runyon Memorial Fund for Cancer Research.

1. Bendich, A., *et al., J. Biol. Chem.,* 1950, v185, 423.

2. Burchenal, J. H., *et al., Cancer,* 1949, v2, 119.

3. Biesele, J. J., *et al., Cancer,* 1951, v4, 186.

4. Stock, C. C., *et al., Annals of N. Y. Acad. Science,* 1950, v52, 1360.

5. Phillips, F. S., and Thiersch, J. B., *Fed. Proc.,* 1949, v8, 325.

6. Cartwright, G. E., *et al., J. Lab. and Clin. Med.,* 1950, v35, 518.

7. Karnofsky, D. A., *et al.,* PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 447.

8. Thompson, R. I., *et al., Science,* 1949, v110, 454.

TABLE I. Effect of Daily DAP Injection on Survival of Mice Infected with RSSE (Pre-virus Treatment).

	Dilution of virus, .05 cc i.p.	No. of animals	Living	Dead	% survival
Control	10^{-3}	20	0	20	0
DAP	10^{-3}	20	0	20	0
Control	10^{-5}	49	4	45	8.2
DAP	10^{-5}	48	15	33	31.2
Control	10^{-6}	65	12	53	18.5
DAP	10^{-6}	63	26	37	41.5
Control	10^{-7}	75	20	55	26.8
DAP	10^{-7}	69	42	27	61

TABLE II. Effect of Daily DAP Injection on Survival of Mice Infected with RSSE (Post-virus Treatment).

	Dilution of virus, .05 cc i.p.	No. of animals	Living	Dead	% survival
Control	10^{-5}	29	2	27	6.9
DAP	10^{-5}	29	9	20	31
Control	10^{-6}	141	26	115	18.4
DAP	10^{-6}	137	58	89	42.5
Control	10^{-7}	45	7	38	15.5
DAP	10^{-7}	46	19	27	41.5

culture of tumor tissue has led to the studies on intact animals here reported.

Materials and methods. Preparation of 2,6-diaminopurine.† The compound was made up daily by dissolving a concentrated suspension in appropriate amounts of saline. The usual amount administered was 70 mg/kg or 1.4 mg/mouse, a dose which had been found to be non-toxic when given daily for 10 successive days. The toxicity of each new preparation of DAP was tested. *Mice.* Animals weighing 18-22 g obtained from commercial breeders were used. No weight loss due to the chemical or to the virus was noted. *Virus.* Source of the RSSE was infected mouse brains maintained in the frozen state until used. A 10% suspension of brain was made in 10% normal horse serum saline and appropriate dilutions were inoculated into both DAP-treated and control animals.

Experimental. Pre-virus treatment. Mice were given 70 mg/kg of DAP in saline by intraperitoneal injection one or 2 days previous to the administration of virus. Groups were then infected by the intraperitoneal inoculation of 0.05 cc of RSSE diluted to 10^{-5}, 10^{-6}, 10^{-7}. Daily injection of DAP was continued until signs of the infection appeared; in most experiments for 10 days. Control animals received comparable injections of saline after infection with the same amount of virus.

A summary of 6 experiments is given in Table I. It is apparent from the results that there were more survivals among the animals which were treated with the DAP than among the controls. The difference is apparent in all 3 groups of animals, those receiving 10^{-5}, 10^{-6} or 10^{-7} dilutions of virus, and is statistically significant.‡ When virus was inoculated at a 10^{-3} dilution, no difference between survival rates of treated animals and untreated controls was noted.

Post-virus treatment. Mice were inoculated with varying dilutions of virus intraperitoneally and treatment was begun 24 hours later by the injection of DAP according to the routine previously described. A summary of 5 experiments is presented in Table II and shows that the DAP, given after virus inoculation, still exerted a significant effect on the survival rates of the infected animals. When treatment was delayed until 48 hours after the inoculation of the virus, no benefit

† This compound was supplied by the Burroughs-Wellcome Laboratories.

‡ The statistical analyses were carried out by Miss Mary MacDonald of the Memorial Hospital Statistical Department.

TABLE III. Distribution of Virus in Animals* treated with DAP Compared with Untreated.

Time after DAP	Blood						Brain					
	Treated			Untreated			Treated			Untreated		
	Pos.	Prob.	Neg.	Pos.	Prob.	Neg.	Pos.	Prob.	Neg.	Pos.	Prob.	Neg.
2 hr	0	1	2	0	0	3	0	0	3	0	0	3
1 day	0	0	3	*2* †	0	2	0	0	3	0	0	3
2	*2*	0	1	*3.5*	0	2	0	0	3	0	0	3
	1.5											
3	*2.5*	0	1	*2*	0	1	0	0	3	0	0	3
	3.5			*2*								
4	*2*	0	1	*2.5*	0	1	0	0	3	*1.5*	0	2
	2			*2.5*								
6	*2*	1	1	0	0	3	0	0	3	*5.5*	1	1
7	0	0	3	0	1	2	*3.5*	0	2	*6.5*	0	1
										2		
	7	2	12	6	1	14	1	0	20	4	1	16

* Animals killed 2 hr after DAP administration.

† Italicized numbers indicate the amount of virus present as determined by intracerebral titration and expressed by the reciprocal of the LD_{50}.

was obtained.

Effect of varying dosage of DAP. When more than 70 mg/kg was given, the compound was not tolerated. When given at 60 mg/kg and if 0.05 cc of a 10^{-6} dilution of the virus was inoculated intraperitoneally, 30% of the treated animals survived, in contrast to 10% of the controls. At lower levels of DAP than this, no therapeutic effect was exerted. When the same amount of DAP was given in divided doses, no additive effect was obtained.

Effect of other routes of administration of DAP. When the DAP was given in daily subcutaneous doses before the intraperitoneally inoculated virus, a slight increase in the survival rate of treated animals over the controls was noted. The figure is not statistically significant. Inoculation of the DAP intracerebrally, followed by inoculation of the virus intraperitoneally 24 hours later, had no effect. The mechanical trauma of repeated intracerebral inoculation was not withstood by the animals. Hence it was impossible to determine if this route of DAP administration was effective. The same difficulty was encountered with intravenous inoculation.

Effect of varying routes of inoculation of the RSSE. The DAP was injected intraperitoneally at 70 mg/kg either before or after virus inoculation. When the virus was given subcutaneously, the same protective effect of the DAP was exerted as when it was inoculated intraperitoneally. When the virus was given intracerebrally the compound was ineffective.

Effect of varying the duration of treatment with DAP. The DAP was administered intraperitoneally at 70 mg/kg for varying periods. Treatment was begun 24 hours after the virus was inoculated. When the DAP was given for only 3 consecutive days, no effect could be observed. When a dose was given every other day for 8 days, the results were as good as if the DAP had been given daily for 7 days. When the compound was administered before or 24 hours after the virus, the treated animals survived approximately one day longer than the control animals. Therefore there appeared to be no significant difference in survival time between the animals treated with DAP before, and those treated after, administration of the virus.

Effect of DAP on other neurotropic virus infections. The DAP had no therapeutic effect against 3 other viruses which infect mice when inoculated intraperitoneally. These are West Nile Encephalitis, Ilheus Encephalitis and Louping Ill. The ineffectiveness of the DAP against the Louping Ill virus was surprising since it is so similar to RSSE that some consider them to be identical. The strain of Louping Ill used in our laboratory differs from the RSSE. The Louping Ill virus is much less infectious when given by the intraperitoneal route than is our RSSE. The DAP was also

TABLE IV. Distribution of Virus in Animals* Treated with DAP Compared with Untreated.

Days after DAP	Blood				Brain			
	Treated		Untreated		Treated		Untreated	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
1	*.5*†	2	*1.5* *1.5* *.4*	0	0	3	0	3
2	*.25* *1.25*	1	*>2* *1.5* *1*	0	0	3	0	3
3	*1* *.7*	1	*3.5* *1.5*	1	0	3	*1.33*	2
4	*1.25* *2.5* *1.5*	0	*3.5* *1.75* *2.5*	0	0	3	*2.4* *4*	1
5	*1* *1.2*	1	*1* *1.75*	1	*4*	2	*3.5* *2*	1
6	*.5*	2	*1.59* *1.5*	1	*7* *4*	1	*3.5* *5.5* *6*	0
7	0	3	*1.5*	2	*8* ‡	2	*5.5* *8* ‡	1
8	0	4	0	4	*8* ‡ *8.5*‡	2	*6* ‡ *8* ‡ *7* *7*	0
9	0	3	0	4	*4* ‡ *8* ‡	2	*8* ‡ *8* ‡	2
10	*.44*	2	0	4	*>9* ‡ *>9* ‡	2	*>9* ‡ *>9* ‡	2
Total	12	19	16	17	10	23	18	15
Toxic	2							

* Animals killed before administration of DAP.

† Italicized numbers indicate amount of virus present as determined by intracerebral titration and expressed as reciprocal of the LD_{50}.

‡ Paralyzed or sick when killed.

ineffective against infection with Lansing poliomyelitis virus and Bunyamwera virus, inoculated intracerebrally.

Distribution of RSSE in treated and untreated animals. In an effort to find out whether there was any different distribution of RSSE in a group of mice treated with DAP from that of an untreated group, 100 mice were inoculated with 0.05 cc of a 10^{-6} dilution of virus intraperitoneally. The following day they were divided into groups of 50 each. One was given daily intraperitoneal injections of DAP (70 mg/kg) and the other of saline. Each successive day 3 mice from each group were killed 2 hours after they had been injected with the DAP. Their brains and bloods were inoculated intracerebrally in normal mice as a test for the presence of RSSE. If any indication of virus was found, the tissues were retested and their virus content established.

The results are recorded as follows: No virus: less than 50% deaths at 10^{-1}. Probable virus: more than 50% deaths at 10^{-1} dilution; and definite virus: virus present after dilution. The results are given in Table III. They show that there was little difference between the two groups in the concentrations of virus in the blood. The absence of virus in the brains of the treated group appears to be significant. The experiment was repeated in the same way except that a group of animals were sacrificed daily on successive days 2 hours before rather than after the injection of the DAP. When the animals became ill, 4 were killed from each group, 2 paralyzed, and 2 apparently healthy. When virus was present, its concentration was titrated (Table IV). In general, the same type of results was obtained. A comparison of the number of animals in the treated and untreated groups showing virus in the blood demonstrated the

following: 19 of 31 (61%) were negative in the treated group (2 were toxic and could not be tested). In the untreated group, 17 of 33 (51%) were negative. Although in the treated group both the number of mice having virus in the blood, and the amounts of virus as determined by titration, were slightly less than in the untreated controls, the differences were not nearly so marked as those found in the brains. Of the treated group, the brains of 23 of 33 animals, or approximately 70%, were negative in contrast to the untreated, in which 33 of 55, or 45.5%, were negative. However, animals of both groups, when paralyzed, showed no significant difference in the amount of virus present in the brains.

Discussion. The effects of treatment with 2,6-diaminopurine of mice infected with Russian Spring-Summer Encephalitis virus have been investigated. The chemical, a substance which is involved in nucleic acid synthesis, has been shown(9) to have an effect on the proliferation of the virus in tissue culture. It has now been demonstrated that DAP exerts a therapeutic effect on animals infected with RSSE even if treatment is begun 24 hours after the virus has been inoculated.

The mechanism of action of the DAP is unknown, but the studies on the distribution of the virus in brain and blood of the treated and untreated animal gives us a clue as to the point of action of the substance.

Since as much virus is present in the blood of the treated animals as the untreated, DAP apparently does not have an effect on the invasiveness of the virus. When the virus is inoculated intracerebrally, the DAP is ineffective, and treated animals which are not protected by the DAP have as much virus in their brains as do the untreated controls. Hence the DAP exerts its activity either on the "blood-brain" barrier or directly on the cells of the brain.

Summary. The compound 2,6-diaminopurine when administered in doses of 70 mg/kg caused an increase in the survival rates of mice infected with Russian Spring-Summer Encephalitis by the intraperitoneal route. It was effective when treatment was started 24 hours after the inoculation of the virus.

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Metabolism of Amines. II: Mitochondrial Localization of Monoamine Oxidase. (19006)

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Despite numerous investigations of its enzymatic function, monoamine oxidase has not been significantly purified. As with other systems that are bound to insoluble particles, the difficulty lies in the extraction of sufficient amounts of active protein for the application of classical methods of fractionation. On the other hand, this difficulty implies a degree of association with organized structures that deserves investigation and suggests that the chemical neighborhood of the enzyme should be explored with fractionations that preserve the integrity of cytological units.

Previous investigators, notably Claude, Hogeboom, Schneider, Dounce, and their collaborators, have recognized the power of morphological fractionation. (See Schneider and Hogeboom(1) for review). In the present investigation it remained to apply their methods and correlate with their results.

Procedure. Adult male rats (170-230 g) of a Sherman strain were used in all experiments. After a week of observation with an unrestricted diet of Purina fox chow, each animal was allowed water during a final period

1. Schneider, W. C., and Hogeboom, G. H., *Cancer Research*, 1951, v71, 1.