ciples, are now in progress. The results here reported are preliminary to a report on a more detailed study of the significance of certain factors in experimental chemical carcinogenesis with carcinogenic hydrocarbons carried out since 1945 (*6*), as well as on the so-called solvent effect for chemical carcinogenesis in general.

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Oxygen Consumption and Radiophosphate Uptake by Minced Brain from Mice of Different Ages in Relation to Propagation of Mouse Encephalomyelitis Virus[1]

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It has been reported previously (*2*) that brain minces from mice up to 9 days of age are capable of supporting the growth of Theiler's GD VII strain of mouse encephalomyelitis virus when cultured in a simple medium containing only salts and glucose; brains from mice 1–2 days of age yield more virus than brains from mice 3–9 days old. No evidence of virus propagation was obtained with brains of mice 10 days old or more. In this study, we investigated the oxygen consumption and radiophosphate uptake of control and virus-infected brain minces from mice of different ages in an effort to determine whether metabolic differences might be associated with the inability of older tissues to support virus propagation.

The methods employed were described in detail in previous papers (*3*, *4*). Minced brain tissue (40–60 mg) was aseptically removed from mice of various ages and added to Warburg vessels or to 50-ml Erlenmeyer flasks containing 2.5 ml of Simms' solution. The pH was adjusted to 9 with dilute NaOH. Cultures were inoculated with virus or control supernatants and brought to a final volume of 3 ml with Simms' solution. Warburg experiments on oxygen consumption were carried out at 35° C with continuous shaking in an atmosphere of air. P^{32} uptake experiments were carried out at 35° C in stoppered, 50-ml Erlenmeyer flasks without shaking and in an atmosphere of air. At the termination of the incubation period all preparations were tested for sterility, and the virus titer was determined by intracerebral injection in mice with serial dilutions as previously described.

TABLE 1
OXYGEN CONSUMPTION BY MINCED BRAIN FROM MICE OF DIFFERENT AGES

Mouse age	No. of exps.	Virus present	Q_{O_2}* Initial	5 hr	12 hr	24 hr	Titer after 24 hr†
1 Day	10	–	90–110	60–80	40–60	30–40	–
	10	+	90–110	60–80	40–60	30–40	10^{-5}
3 Weeks	3	–	130–170	60–90	10–30	0	–
	3	+	130–170	60–90	10–30	0	10^{-2}
Adult	4	–	180–220	40–60	0	0	–
	4	+	180–220	40–60	0	0	10^{-2}

* Values of Q_{O_2} are expressed as µl O_2/hr/100-mg wet weight and show the ranges for the different experiments.

† The highest tenfold dilution that killed at least half of a group of 7 mice when 0.03 ml was injected intracerebrally. A titer of 10^{-2} represents only survival of the original virus added.

Oxygen consumption was measured on control and virus-infected tissues using the direct method of Warburg as previously described (*3*).

The results of experiments with minced brain from 1-day-old mice, 3-week-old mice, and adult mice are given in Table 1. The initial metabolic rate is distinctly higher in 3-week-old and in adult mouse brain than in the 1-day-old group. However, the metabolism of the older tissue declines much more rapidly with time than does that of 1-day-old brain mince. As was previously noted (*3*), the presence of the virus had no influence on oxygen consumption of the 1-day-old mouse brain, although the virus was shown to propagate rapidly in this tissue. No virus propagation was observed in 3-week-old or adult mouse brain.

Studies were made of the uptake of radioactive orthophosphate into the organic acid-soluble (OAS) fraction, the phospholipide (LP) fraction, and the "total protein-bound" (TPP) fraction by the procedures previously described (*4*). The chemical analysis for P^{32} was carried out by a modification of the method of Fiske and Subbarow (*1*) in which ascorbate was used as a reducing agent and a heating period was employed for color development and stabilization. Radioactive samples were prepared in ¼-oz tin ointment dishes, and

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TABLE 2

P^{32} UPTAKE INTO THE OAS, LP, AND TPP PHOSPHATE FRACTIONS OF MINCED MOUSE BRAIN OF VARIOUS AGES INCUBATED 24 HR IN THE PRESENCE AND ABSENCE OF VIRUS

Mouse age, days	Relative specific activity*						Virus† titer
	OAS		LP		TPP		
	No virus	Virus	No virus	Virus	No virus	Virus	
1	53.5	51.7	2.9	4.2	2.4	3.5	10^{-5}
	47.6	50.8	3.0	4.1	2.3	3.5	
7	22.2	30.2	2.0	2.2	1.2	1.5	10^{-4}
	26.0	24.3	1.9	2.2	1.3	1.6	
10	19.4	24.8	1.2	1.2	0.9	0.9	10^{-2}
	21.3	24.2	1.4	1.4	0.9	0.9	
14	26.0	24.8	0.8	0.8	0.7	0.7	10^{-2}
	23.0	24.7	0.8	0.8	0.7	0.7	
23	19.6	20.1	0.6	0.6	0.6	0.6	10^{-2}
	18.5	17.4	0.6	0.6	0.6	0.6	

* Relative specific activity $= \frac{\text{counts}/\mu g\ P^{31}\ \text{in the fraction}}{\text{counts}/\mu g\ P^{31}\ \text{in IP fraction}} \times 100$.

† A titer of 10^{-2} represents the initial virus inoculum and corresponds to survival only.

counted with a thin, mica-window Geiger-Müller counter tube. A minimum of 3,000 counts per sample was taken.

Table 2 gives data for the uptake of P^{32} in a 24-hr incubation period into the OAS, LP, and TPP fractions of virus-infected and noninfected minced brain from 1-, 7-, 10-, 14-, and 23-day-old mice. Values are expressed as relative specific activities, i.e., the specific activity of the fraction relative to the specific activity of the inorganic phosphate fraction (IP). It is apparent that the incorporation of P^{32} into the lipide and protein-bound fractions was most extensive in the 1-day-old minced mouse brain, and was less extensive as tissue from older mice was employed.

The previously noted stimulation of P^{32} uptake by virus infection of minced 1-day-old mouse brain (*4*) is apparent in the experiments reported in Table 1. A definite but smaller stimulation is apparent in the 7-day-old mouse brain. P^{32} uptake by brain from mice older than 7 days did not show stimulation by virus infection. Stimulation of P^{32} uptake by virus infection is seen to correspond to the propagation of the virus. No difference between the phosphorus distribution in the control and infected cultures was noted, although there was the expected increase in the total lipide and protein phosphorus in older tissue (Table 3).

The initial rate of oxygen consumption of minced brain from 1-day-old mice is considerably lower than tissue from 3-week-old or adult mice. The 1-day-old tissue, however, can maintain its oxygen consumption

TABLE 3

DISTRIBUTION OF PHOSPHORUS IN THE VARIOUS FRACTIONS OF MINCED MOUSE BRAINS OF DIFFERENT AGES INCUBATED 24 HR IN THE PRESENCE AND ABSENCE OF VIRUS

Mouse age, days	Micrograms of phosphorus per 100 mg wet tissue					
	OAS		LP		TPP	
	No virus	Virus	No virus	Virus	No virus	Virus
1	25	31	44	48	45	46
	24	25	47	43	44	45
7	26	30	55	55	43	45
	28	24	56	58	45	44
10	30	26	54	53	48	49
	25	24	52	51	49	47
23	30	25	64	60	57	53
	28	29	61	60	55	56

for long periods in a culture medium containing only salts and glucose, whereas the Q_{O_2} of tissue from older mice declines much more rapidly. The propagation of Treiler's GD VII virus in minces of 1-day-old mouse brain did not affect the rate of oxygen consumption.

In a 24-hr incubation period, the minced brain tissue from 1-day-old mice was found to incorporate radioactive phosphate into the tissue lipides and proteins more extensively than the tissue from older mice. The virus did not grow in minced brain cultures from mice older than 9 days and exerted a stimulatory effect on the uptake of P^{32} into the lipides and proteins only in those young cultures in which virus propagation could be demonstrated. The data suggest that the ability of 1-day-old minced mouse brain cultures to permit the propagation of the virus is associated with the ability of this tissue to maintain its oxidative metabolism over a sufficiently long period to allow virus multiplication. Most virus production appears to occur in the period from 12 to 24 hr (*2*).

The difference in the ability of 1-day-old mouse brain and of older mouse brain to support the propagation of Theiler's GD VII virus *in vitro* may be associated with the greater ability of the younger mouse brain to maintain oxidative or glycolytic metabolism for long periods of time. This hypothesis would resolve discrepancies and explain the observation that the intact adult mouse develops paralysis in spite of the failure to demonstrate virus propagation *in vitro* in adult brains.

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