Oxidation of ^{14}C-Glycerol Administered by Ventriculocisternal Perfusion in the Dog[1] (36971)

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The question of the extent to which substrates other than glucose are oxidized by the brain has engaged the attention of numerous investigators (1). Evidence has appeared that glycerol may be such a substrate in man (2), in the guinea pig (3), and in the rabbit (4). Since the technique of ventriculocisternal perfusion has proved valuable in studying the metabolism of free fatty acids (5), and β-hydroxybutyrate (6), by cerebral tissue, it seemed to us that it would be of interest to investigate the fate of glycerol administered by this route.

Methods. Four mongrel dogs, within the weight range of 20–28 kg, were used for these experiments, food having been withheld for about 18 hr prior to the experiments. The animals were anesthetized with sodium pentobarbital (30 mg/kg), and maintained in reasonably constant depth of anesthesia by a continuous infusion of the same drug (4 mg/kg/hr). A tracheal tube was surgically inserted and artificial respiration was provided by a pump throughout the experiments, at the rate of 360 ml/kg/min. The heads of the dogs were then positioned in a frame immobilizing the skull for the insertion of cannulas.

A ventriculocisternal perfusion system was set up by a technique based on that of Carmichael, Feldberg, and Fleischhauer (7), and the details are described in a preceding paper (5). The artificial CSF consisted of a glucose-free modified Krebs–Henseleit solution (8), containing 400 μmoles glycerol/ liter, with 1-3-^{14}C-glycerol added in sufficient amount to give approximately 250,000 dpm/ml. The ^{14}C-glycerol was purchased from New England Nuclear. Prior to its introduction into the ventricle the artificial CSF was equilibrated with 5% CO_2, 15% O_2, and 80% N_2, and was maintained in contact with a similar gas mixture throughout the period of perfusion. It entered the ventricle at the rate of 0.494 ml/min and outflowing fluid was collected every 10 min, alternate collections being made under mineral oil to minimize loss of CO_2. At 95, 135, and 175 min simultaneous blood samples were taken from the femoral artery and the confluens sinuum into chilled heparinized tubes.

CO_2 determinations were performed on blood and ventricular effluent using the Technicon AutoAnalyzer and $^{14}CO_2$ was measured by the method of Passman, Radin and Cooper (9). Glycerol was estimated by the enzymatic procedure of Wieland (10), and plasma glucose by the AutoAnalyzer method based on the reduction of ferricyanide.

Counting of samples was performed in a Packard liquid scintillation spectrometer and the results are expressed as dpm after correcting for quenching and efficiency of counting. The purity of the ^{14}C-glycerol in the infused fluid was checked by paper chromatography using butanol–acetic acid and water (40:10:50) as the developing solvent. No radioactivity was found in the glucose.

Radioactivity other than $^{14}CO_2$ in the blood samples was measured by mixing 0.1 ml of acidified plasma with a water miscible scintillator (11), and counting directly in the Packard scintillation spectrometer.

Results. Table I shows the radioactivity present as $^{14}CO_2$ in the outflowing perfusate compared with that in arterial and venous

[1] Supported by Grant HE 03130 from the National Institutes of Health.

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TABLE I. $^{14}CO_2$ Concentration of Blood and Perfusate in the Course of Ventriculocisternal Perfusion of Glycerol (dpm/ml).[a]

Dog no.	Perfusion (min)								
	95			135			175		
	A	V	P	A	V	P	A	V	P
1	121	872	1826	131	1147	2015	140	946	1793
2	77	163	1577	89	187	1745	95	230	1874
3	22	109	715	27	121	744	26	89	784
4	35	237	950	32	192	960	30	165	1015

[a] A = arterial blood; V = blood from confluens sinuum; P = perfusate.

blood, at three time intervals from the initiation of perfusion. As shown, the counting rate is substantially higher in the perfusate than in either blood sample at all times and in all experiments.

In Table II, the specific activity of the CO_2 in the same fluids is compared and again the values in outflowing perfusate exceed those in both arterial and venous blood in every instance.

Table III indicates the rate of oxidation of glycerol introduced into the ventricular system. The method of calculating this value is indicated at the bottom of Table III. Note that in the first experiment this rate appeared to be very high while in the remaining three it was considerably lower, although it remained relatively constant for a given animal throughout the run. The cerebral blood flow assumed in making the calculations on which Table III is based (25 ml/min), was that found in other dogs under the same experimental conditions as those employed in the present study (5).

Table IV shows the proportion of the infused ^{14}C-glycerol which was recovered in the effluent at various times during the experimental runs, and so gives an estimate of the proportion removed by the intracranial structures. As in Table III, a striking difference is evident between the findings in Dog 1 and those in the remaining three in the series. However, in all instances, an appreciable removal of glycerol was demonstrated.

The arteriovenous differences for glucose presented in Table V are intended to demonstrate that the brains of our animals were metabolically active. The data do, however, also reveal that there was not a one-to-one relation between the uptake of glucose carbon and the release of carbon dioxide into the blood and perfusate. This could well be due to the production of lactic acid.

Discussion. The experiments described demonstrate that glycerol introduced into the ventriculocisternal space of the dog can be oxidized to CO_2. As in previous studies, utilizing ventriculocisternal perfusion, these results do not prove unequivocally that brain tissue was the site of the oxidation. It can be calculated from Table IV that in Dogs 2, 3, and 4, about 10% of the perfused counts

TABLE II. Specific Activity of Blood and Perfusate CO_2 in the Course of Ventriculocisternal Perfusion of Glycerol (dpm/μmole).[a]

Dog no.	Perfusion (min)								
	95			135			175		
	A	V	P	A	V	P	A	V	P
1	18.06	89.90	139.39	17.95	117.04	149.26	18.92	92.75	136.87
2	8.56	13.81	91.16	8.99	15.85	102.65	9.13	17.56	112.22
3	1.54	6.41	15.70	1.82	6.61	15.83	1.81	5.39	17.20
4	2.41	13.78	38.78	2.29	11.50	39.51	2.07	10.65	42.47

[a] A = arterial blood; V = blood from confluens sinuum; P = perfusate.

TABLE III. Oxidation of Glycerol in the Course of Ventriculocisternal Perfusion of Glycerol (nmole/min).

Dog no.	(min): 95	135	175
1	30.99	41.58	33.13
2	2.16	2.44	3.15
3	4.40	4.69	3.43
4	10.14	8.22	7.13

Formula used to calculate the oxidation of glycerol:

$$\text{Amt. of glycerol oxidized} = \frac{\text{V—A difference of } {}^{14}CO_2 \times 25^a}{\text{sp act of glycerol in perfusion fluid}} + \frac{{}^{14}CO_2 \text{ in perfusate} \times 0.494^b}{\text{sp act of glycerol in perfusion fluid}}$$

[a] Assumed rate of cerebral blood flow (ml/min).
[b] Rate of perfusion (ml/min.)

disappeared, which amounts to some 14,000 dpm/min. Of the counts which disappeared some 20% can be accounted for as $^{14}CO_2$. If the rest of the counts entered the sinus blood, one would expect an arteriovenous difference of 450 dpm/ml. Since the arteriovenous difference in non-CO_2 counts was insignificant, we were led to conclude that a proportion of the missing counts had been retained by some intracranial structure. With this in mind, brain samples from several regions were taken at the end of the last three experiments, extracted with Folch reagent (12), and the extracts were chromatographed on thin layer plates. The radioactivity in the various lipid classes was measured and significant counts were found in the phospholipid fraction. The extracts were processed in only a semiquantitative way and, in view of this together with probability of wide regional variations, no estimate of the total incorporation of glycerol carbon into the brain lipids has been attempted.

Previous work using the rabbit has shown that when glycerol is perfused through the ventricles, some penetration into the brain occurs (13), and it therefore, seems probable that some of the oxidation seen in the present experiments was taking place within the brain substance.

Whereas older work, relying on classical biochemical techniques (14), failed to demonstrate oxidation of glycerol by brain slices, the more recent experiments of Sloviter and Suhara (4), using isotopically labeled glycerol did show measurable production of labeled CO_2. Further evidence for the participation of glycerol in brain metabolism comes from the studies of Seiler, Moller and Werner (15), who found substantial incorporation into amino acids from ^{14}C-glycerol.

It is conceivable that the oxidation which we have observed is in fact taking place either at the ependyma, or in the membranes enclosing the subarachnoid space and this is a possibility which must be taken into account when interpreting data obtained either *in*

TABLE IV. ^{14}C-Glycerol Recovered from Outflowing Perfusate as a Percentage of ^{14}C-Glycerol Perfused in Inflowing Perfusate During Ventriculocisternal Perfusion of Glycerol.

Dog no.	Perfusion (min) 95	135	175
1	49.87	52.72	42.15
2	88.28	89.08	93.12
3	90.14	83.57	83.47
4	88.27	84.97	87.80

TABLE V. Glucose in the Course of Ventriculocisternal Perfusion of Glycerol (μmole/ml).[a]

Dog no.	Perfusion (min) 95 A	95 V	95 A—V	135 A	135 V	135 A—V	175 A	175 V	175 A—V
1	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	5.89	5.12	0.77	5.94	5.23	0.71	6.00	5.56	0.44
3	6.77	5.94	0.83	7.04	6.33	0.71	7.26	6.33	0.93
4	5.56	5.06	0.50	5.78	5.39	0.39	6.11	5.67	0.44

[a] A = arterial blood; V = blood from confluens sinuum; NA = not available.

vivo, or from superficial slices. On the other hand, it now seems likely that glycerol, β-hydroxbutyrate (6), and free fatty acids (5), once they have gained access to the brain, undergo some oxidation and may, under certain circumstances, serve as supplementary oxidizable substrates for the organ. Convincing evidence has recently appeared (16) which indicates that the brain is capable of metabolic adaptation on a substantial scale. It follows therefore, that a substrate such as glycerol, which is oxidized in only very small quantities in the brain of an animal in normal nutritional and hormonal status, may be utilized to a greater extent under other conditions. The possibility of such adaptive changes clearly adds potential significance to even slight oxidative activity with respect to a blood-borne substrate.

Summary. 1-3^{14}C-Glycerol in artificial CSF was perfused from the third ventricle to the cisterna magna in dogs. The appearance of $^{14}CO_2$ in the perfusate and in blood from the confluens sinuum was studied after 95, 135, and 175 min of perfusion. Specific activity of CO_2 in the perfusate at all times exceeded that of the venous blood which in turn exceeded that of arterial blood. The calculated rate of oxidation of glycerol was between 2 and 10 nmoles/min in the majority of animals. The disappearance of counts from the perfusate indicated a consistent uptake of the labeled glycerol. A significant fraction of the labeled glycerol was recovered in the perfused tissue.

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Received Sept. 14, 1972. P.S.E.B.M., 1973, Vol. 142.