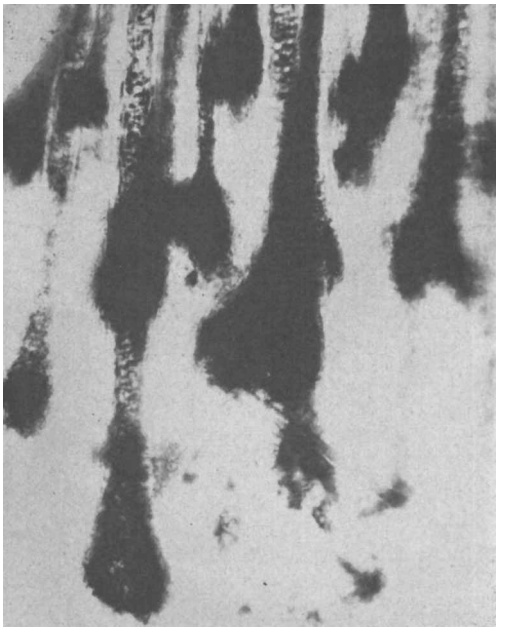

FIG. 3. Whole mount of brown skin fragment in the presence of tyrosine (experimentals). Note the very densely pigmented hair bulbs and lower hair portions. Note also upper hair portions, showing the same pigmentary details as found in the control hairs.

Thus, presumably for the first time, are demonstrated both the enzymatic oxidation of, and pigment-formation from, tyrosine, in the same sample of normal mammalian skin.

Discussion. The positive results reported here strongly indicate that at least some of the dark mammalian hair and skin pigments are products of the tyrosinase system. Studies on a rather extensive series of guinea-pig coat-color genotypes fail, however, to reveal any simple parallelism between tyrosinase or dopa oxidase activity and genetically determined amount or kind of natural pigment(4). These studies, which will be reported and considered in detail elsewhere, confirm in certain major respects previous studies of dopa reactions in the case of guinea-pig skin(5,6).

I am indebted to Professor Sewall Wright for suggestions and criticisms and providing genetic material, bred and identified by him; to Professor Hewson H. Swift, for help in histological technic and photomicrography; and to Professors Benson Ginsburg and Dorothea S. Miller, for laboratory facilities.

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Cultivation of Lansing Poliomyelitis Virus in Tissue Culture. II. Utilization of Glucose in Synthetic Medium.* (19496)

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(Introduced by R. C. Parker.)

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The work of Enders and his associates (1-3) has indicated that poliomyelitis virus can be propagated in cultures of human embryonic tissues. In their work, Hanks-Simms' fluid was used as the source of nutrient. Recently, we have shown(4) that this fluid can be replaced by a synthetic nutrient medium, mixture No. 199, devised by Morgan, Morton, and Parker(5,6). Mixture 199 is of chemically defined composition, and allows of survival of suspended tissue fragments and proliferation of virus for a prolonged period. Hitherto, metabolism has been followed by observing changes in pH. Enders, for example, has reported that the pH values of uninfected cultures are lower than those of infected cultures. Since the Meyerhof sequence of glycolysis has been found to apply to almost all biologic systems, it seemed appropriate to

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study the utilization of glucose in infected and uninfected cultures, in the belief that the state of metabolism could be determined with a high degree of sensitivity.

Methods. Three experiments on the metabolism of tissue cultures infected with Lansing virus have been carried out so far. In each experiment, brain and cord was obtained from a different human embryo. The technic was that described by Weller and Enders(7), and used in our previous study (4). The tissue was minced and distributed between 25 ml Erlenmeyer flasks (8 for each series); mixture 199 was used as the source of nutrient. Hanks-Simms' medium was also used in Exp. 1 for comparative purposes. In Exp. 1 and 3, the source of virus was 0.1 ml of a 10% suspension of mouse central nervous system infected with the Lansing strain of poliomyelitis virus. In Exp. 2, the virus inoculum consisted of 0.1 ml of the 14th culture fluid removed in Exp. 1. In the 3 experiments, virus suspension was added to 4 of the Erlenmeyer flasks, leaving the remaining 4 to serve as uninoculated controls. The culture fluid was removed and replaced with fresh medium every 3-5 days, or when the pH fell to 6.8. Mouse infectivity tests were carried out by the cerebral inoculation of 0.03 ml of pooled culture fluids. Glucose estimations were carried out on pooled uninfected and pooled infected culture fluids in Exp. 1 and 2, and on individual culture fluids in Exp. 3. Glucose estimations were carried out according to the method of Nelson(8). The glucose level of the media used was about 100 mg%. The results are expressed in terms of % glucose utilization.

Results. Exp. 1. The results are shown in Table I. It will be seen that glucose was utilized by the tissues in the Hanks-Simms' medium for 29 days, but in the 199 synthetic mixture series it was still being utilized, although at a low level, when the experiment was terminated at 78 days. It will also be noted that glucose utilization was lower in both series of infected cultures than in the control uninfected cultures. Infectivity tests in the Hanks-Simms' series showed that there were only isolated deaths among mice inoculated with culture fluids later than the 7th. However, in the 199 series, deaths occurred in mice inoculated with all culture fluids up to and including the 16th.

TABLE I. Glucose Utilization and Infectivity Tests in Cultures of Human Embryonic Brain and Cord Infected with Lansing Poliomyelitis Virus.*

Culture fluid	Incubation, days	% glucose used: Uninfected	% glucose used: Infected	Mouse† deaths
	Hanks-Simms' medium			
1	2	54.2	53.4	5/6
2	6	48.6	42.6	2/6
3	10	43.4	33.3	6/6
4	13	31.4	21.9	5/6
5	17	23.6	9.4	3/6
6	21	4	.0	4/6
7	25	15.7	3	3/6
8	29	8.8	2.2	1/6
9	33	.0	.0	1/6
10	38	.0	.0	0/6
11	42	.0	.0	1/6
12	46	.0	.0	0/6
13	50	.0	.0	0/6
14	54	.0	.0	0/6
15	59	.0	.0	0/6
	Synthetic mixture 199			
1	5			6/6
2	9	56.3	47.3	5/6
3	13	58	31.8	6/6
4	16	57.5	29.4	6/6
5	20	62	29.9	5/6
6	23	44	28	5/6
7	27	37.6	21.7	2/6
8	32	47.7	28.2	6/6
9	36	29.1	13	5/6
10	40	29.5	14.8	6/6
11	44	5.4	.0	6/6
12	48	22.7	14.3	4/6
13	53	8.5	1.9	3/6
14	57	8.5	2.9	5/6
15	61	7.3	2.3	6/6
16	65	8.7	3.1	3/6
17	69	5.9	.0	0/6
18	74	11.5	7.1	0/6
19	78	5.2	.0	0/6

* Cultures inoculated with 500 LD_{50} (for mice) of Lansing virus mouse pool.

† Mice inoculated cerebrally with .03 ml of pooled infected culture fluids.

Exp. 2. The results are shown in Table II. It can be seen that the amount of glucose used is similar in infected and uninfected cultures for the first 3 pairs of culture fluids; the characteristic differences appeared subsequently. It was noted that from about the 20th day the infected tissue fragments underwent marked degeneration, resulting in a very turbid medium. This would appear to be due to the cytopathogenic effect(3).

TABLE II. Glucose Utilization and Infectivity Tests in a First Subculture of Lansing Virus in Human Embryonic Brain and Cord, with Synthetic Mixture 199.*

Culture fluid	Incubation, days	% glucose used		Mouse deaths
		Uninfected	Infected	
1	3	94.8	90.6	1/5
2	7	76.4	84.8	6/6
3	11	87.6	92.8	6/6
4	15	90.4	44.7	6/6
5	19	74.8	20.3	6/6
6	22	55.3	12.8	6/6
7	26	56.7	9	5/6
8	29	35.2	6.1	0/6
9	33	30.5	6.5	0/6
10	36	20.2	3.9	0/6
11	40	13	5	0/6

* Cultures inoculated with .1 ml of the 14th culture fluid of Exp. 1 (Mixture 199).

Exp. 3. The results are shown in Table III. It will be seen that during the first 3 days the amount of glucose used by the tissues is about the same in infected and uninfected cultures. Thereafter, the values for glucose utilization fell more rapidly in the infected than in the uninfected cultures. Thus, the amount of glucose used in these cultures followed the same general pattern as that seen in the previous experiments.

Discussion. It has been shown, in confirmation of our previous work, that the Lansing poliomyelitis virus proliferates in cultures of human embryonic brain and cord supplied with synthetic nutrient mixture 199, devised by Morgan, Morton and Parker(5). Tests for glucose utilization in culture fluids have shown that these values are significantly lower in cultures containing virus than in the uninfected control cultures. The apparent indications are that when glucose utilization falls to a low level, the liberation of virus into the culture fluids, as shown by infectivity for mice, also falls. It is suggested that tests for glucose utilization are of value in studies of the growth of Lansing virus in tissue culture, as they give an indication of the metabolic state of the tissue in which the virus grows. In this connection, it is of interest to recall that Racker and Krimsky(9) showed an inhibition of glucose utilization in homogenates of brains of mice infected with Lansing virus.

Ackermann has postulated that virus is formed in infected tissue due to an abnormal sequence of some normal metabolic cellular process, and that the functioning of the Krebs cycle is essential for the propagation of influenza virus(10). He also found that dl-ethionine and dl-methionine prevented the propagation of influenza PR8 virus and Lansing poliomyelitis virus, and that this inhibition could be overcome by addition of l-methionine(11,12).

It appears possible that the proliferation of Lansing virus in fragments of human embryonic tissues can be studied when these tissues are suspended in a synthetic nutrient medium, and that metabolic studies may be made by addition or removal of the component parts

TABLE III. Glucose Utilization and Infectivity Tests in Cultures of Human Embryonic Brain and Cord Infected with Lansing Virus, with Synthetic Mixture 199.*

Culture fluid	Incubation, days	% glucose used								Mouse deaths
		Uninfected				Infected				
1	3	86.8	95.5	65.3	91.4	87.2	91.7	86.2	90.9	6/6
2	7	61.4	36.1	43.5	58.2	43.6	52.2	47.4	46.2	6/6
3	11	62.7	53.8	32.4	49.5	21.1	31.2	35.9	28.1	5/6
4	15	49.7	48.2	30.2	50.5	14.1	20.7	16	17.5	4/6
5	19	46.1	41.7	29.2	46	9.3	18.7	14	9.3	5/6
6	24	51.5	46.5	26.7	48.5	7.9	18.9	13.4	4.5	3/6
7	29	35.8	25.7	21.8	37.6	.0	8	1.5	.7	3/6
8	32	28.2	16.7	13.4	33.7	.0	9.3	2.3	2.3	4/6
9	37	41.7	20.7	31.7	50.7	3.8	18.4	14.2	5.1	0/6
10	42	31.3	15.2	21	40.3	.0	7.3	.8	.0	5/6
11	47	22.8	3.6	10	33.8	.0	4.4	.6	.0	4/6
12	51	15.8	6.7	10.1	23.6	2.3	8.7	6.7	1.5	4/6
13	55	13.4	6.5	5.8	19.4	4.6	8.7	3.1	3.1	2/6
14	58	8	2.2	.8	8.7	.0	.8	.0	1.5	1/6
15	62	3.4	.0	.0	9.1	.0	.4	.0	.4	1/6

* Cultures inoculated with 500 LD_{50} (for mice) of Lansing virus mouse pool.

of this medium.

Summary. 1. Lansing poliomyelitis virus proliferates in cultures of human embryonic brain and cord supplied with a synthetic nutrient medium. 2. The amount of glucose used by cultures that contain virus is significantly less than that used by uninoculated control cultures.

The human embryos used in these studies were obtained through the courtesy of the gynecological staff of the Toronto General Hospital. We are indebted to Dr. R. C. Parker and his colleagues for supplies of the synthetic medium.

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Cultivation of Coxsackie Virus in Embryonated Eggs and in Chick Tissue Cultures. (19497)

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First attempts to grow Coxsackie virus in embryonated hens' eggs were unsuccessful(1). Since then there has been only one report of adaptation to growth in eggs. Huebner(2), after alternate mouse to egg passage, carried a Group A, Type 2 strain through a number of consecutive yolk-sac passages. Similarly, there has been a report of successful cultivation in tissue culture. Slater and Syverton(3) maintained a Group A, Type 4 strain in embryonic mouse cell medium for 24 passages.

Results of the following investigation confirm the difficulty of growing the Coxsackie viruses in embryonated eggs and suggest that, while Group A, Type 2, may perhaps be more readily adapted than other types, the individual strain appears to be the most important factor.

Methods and materials. Virus strains. Thirty-one strains were used, including representatives of 10 types of Group A and 3 of Group B. In most cases, 2 or more strains of a given type were tested but only one strain each of Group A, Type 6, 7, and 9 and Group B, Type 2. Twelve strains had been received from other laboratories as muscle suspension or mouse brain; the remainder were isolated in this laboratory from human fecal specimens. Stock supplies of the virus were maintained as infected mouse brain in 50% glycerol. Infected leg tissue suspension was the usual material for egg and tissue-culture inoculation, the exceptions being 2 Group B strains, where brain tissue was used as the inoculum.

Embryonated eggs. After preliminary exploration, the following procedure was used as a screening test. Eggs were incubated for 7 days at approximately 37°C; 0.1 ml of 10% virus suspension was inoculated into the yolk sac and incubation continued for 6 days at approximately 35°C before harvesting embryos and a portion of the yolk-sac tissue. The heads, minus the eyes, of 6 to 8 embryos were weighed, ground with sterilized sand, and a 20% suspension prepared, using as a diluent 0.85% saline containing 10% beef infusion broth. The suspensions were centrifuged for