LASTING BIOLOGICAL EFFECTS OF EARLY ENVIRONMENTAL INFLUENCES*

II. Lasting Depression of Weight Caused by Neonatal Contamination

By EGILDE SERAVALLI,‡ Ph.D., and RENÉ DUBOS, Ph.D.

(From The Rockefeller University, New York 10021)

Plates 90 and 91

(Received for publication 18 December 1967)

Infections occurring in utero or shortly after birth can have manifestations extending throughout the life-span of the affected organism. These manifestations range from stunted growth or malformations to immunological paralysis or infection immunity. It will be shown in the present paper that a lasting depression of weight can be readily and consistently induced by contaminating specific-pathogen-free[1] mice shortly after their birth with an agent derived from the intestinal tract of so-called "normal" mice.

The phrase "normal mice" is used here to denote animals that have been raised under ordinary conditions and appear healthy. The weight-depressing agent[2] to be discussed in this paper has been recovered from the three colonies of ordinary albino mice tested for its presence. As it can be readily transmitted by contact to newborn mice of certain SPF colonies without causing in them any obvious disease other than weight depression, such experimental transfer will be referred to as contamination rather than infection.

The transfer of the growth-depressing effect was first observed 5 yr ago while comparing the characteristics of two related mouse colonies, both of them maintained at that time in our laboratory (2). Animals of the Standard Swiss (SS) colony were produced on a large scale without any special precaution, whereas the animals of the NCS colony which had been derived from SS as described in preceding papers (1) were maintained under protected conditions.

Despite their common origin, the SS and NCS mice differed in many char-

* These studies were supported (in part) by the U.S.P.H.S. Grant No. AI 05676 and the Health Research Council of the City of New York Research Project No. U-1049.

‡ Present address: Istituto Nazionale per lo Studio e la Cura dei Tumori, Piazzale Gorini 22, Milano, Italy.

[1] As stated in the preceding paper (1), the designation SPF does not necessarily imply that the animals are completely free of potential pathogens.

[2] Although the word "agent" will be used in the singular throughout this paper, we realize that the experiments described here do not rule out the possibility that weight depression can be caused by more than one filterable agent.

acteristics. Of particular relevance for the present report was the fact that NCS animals were much heavier at weaning time than the SS animals, and achieved a greater adult weight, irrespective of the diet used. This difference in growth appeared to be related to differences in indigenous flora.

The intestinal flora of NCS animals always contained fewer microbial species than the SS flora and was free of certain potential pathogens. The qualitative differences in the composition of the intestinal flora suggested the hypothesis, now to be documented, that the growth of ordinary mice is commonly retarded by the activities of some microbial agent commonly present in their intestines. The findings to be reported here indicate that at least one of the agents responsible for weight depression can readily pass through filters that hold back the usual bacterial species.

Materials and Methods

Animals.—Mice of two SPF colonies, namely NCS and COBS, were used in the present study. The origin of these colonies has been described in the preceding publication (1).

As the SS colony has been discontinued at The Rockefeller University, other animals originally derived from this colony were obtained from commercial farms; they will be designated "ordinary" mice. Intestinal material derived from SS and ordinary adult mice which were seemingly healthy was used to contaminate SPF animals (either NCS or COBS).

The litter size was reduced to eight within 1 or 2 days after birth whenever the numbers of young born exceeded this figure; the litter was maintained at six or seven in the rare cases when only this number of young were born; smaller litters were discarded.

Experimental Contamination.—Contamination was practiced 2 days after birth except when otherwise indicated in the text. The whole intestinal tract of SS or ordinary mice was homogenized with Teflon grinders, using 5 ml of Tris-buffered salt solution per animal. The pooled suspensions from three mice were centrifuged at 3000 rpm for 30 min at 0°C. In early experiments, the whole suspension was used as contaminating material. In experiments carried out during the past 2 yr, intestinal homogenates were passed through Millipore filters before use. The filters used were of 0.8, 0.45, 0.22, and 0.10 μ porosity.

Intestinal homogenates and filtrates were similarly prepared from SPF mice which had been contaminated with the intestinal material described above or with tissue cultures to be described later.

The presence of bacteria and PPLO organisms was tested by inoculating filtered homogenates or tissue culture on a variety of appropriate culture media that were incubated aerobically and anaerobically.

Newborn SPF mice were contaminated by introducing into their mouth two small drops (each containing approximately 0.025 ml) of the material to be tested, using a disposable syringe with a blunt needle (26⅜ gauge).

Dilutions of the contaminating material were prepared in Tris-buffered salt solution as indicated in the text. It should be mentioned at this point that administration of sterile Tris solution per os to newborn mice did not affect their growth rate.

Tissue Cultures.—Of the several types of tissue cultures tested, only two have given positive results with the filtrates of intestine homogenate under consideration, namely BHK-21 (a line of baby hamster kidney cells) and mouse embryo primary culture. BHK-21 was propagated in 16 oz prescription bottles in a medium consisting of basal medium Eagle with Earle's balanced salt solution (EBME) and 5% calf serum inactivated for 30 min at 56°C (growth

medium). After 2 days of incubation at 37°C, the medium was removed and replaced with 1.5 ml EBME medium containing inactivated calf serum in a final concentration of 2% (maintenance medium).

Mouse embryo cell tubes were obtained from GIBCO Laboratories (Grand Island Biological Company, GIBCO, Grand Island, New York) every week and, as soon as received, were placed overnight at 37°C. The next day the medium was replaced by 1.5 ml of EBME to which was added inactivated horse serum in a final concentration of 5% (maintenance medium).

Prepared tissue culture media, sera, and trypsin were all obtained from GIBCO. A mixture of penicillin (100 units/ml) and streptomycin (100 μg/ml) was added to the media.

BHK-21 and mouse embryo cells were infected with 0.3 ml of the filtrate of homogenized intestines diluted 10^{-1}, 10^{-2}, and 10^{-3} in Tris solution after the medium had been removed from the tubes. The inoculum was left in contact with the cells for 30 min at 37°C, then it was removed and replaced by 1.5 ml of maintenance medium.

Weekly passages were accomplished after the cells had been detached from the tube surface with glass beads. The cells were examined microscopically three times weekly.

RESULTS

Depression of Weight Produced by Contaminating Newborn SPF Mice with Material Derived from Intestinal Homogenates of Ordinary Mice.—As mentioned earlier, mice of both the NCS and COBS colonies are heavier at weaning time than SS or ordinary mice fed the same diet; furthermore, they become larger adults. All the mouse colonies used in the present study originate from the same original stock of so-called Swiss mice and are therefore genetically similar (3). Despite this similarity of origin, it might be assumed that the more rapid growth of the SPF animals derived from SS mice might have been the outcome of genetic mutations. This is rendered unlikely, however, by the fact that SPF mice (NCS or COBS) lose all their distinctive characteristics when they are contaminated at birth with the intestinal contents of ordinary mice.

The fact that SPF mice contaminated at birth grow at a slower rate than uncontaminated controls was first demonstrated by using the intestinal content of SS mice as contaminating material (2). A similar phenomenon has now been confirmed repeatedly by contaminating NCS and COBS mice with intestinal contents from ordinary mice.

Since NCS and COBS mice have a simpler intestinal bacterial flora than ordinary mice, it appeared possible that certain bacterial species present in the latter animals but not in the former were responsible for the difference in growth rates. To test this hypothesis, some 20 bacterial strains were isolated from the intestinal content of ordinary mice and cultures of these bacteria were used to contaminate newborn NCS mice by the oral route.

The cultures of coliforms (rapid lactose fermenters and slow lactose fermenters) multiplied rapidly throughout the whole gastrointestinal tract and persisted at very high levels (approximately 10^9 living bacteria per g of tissue) almost until the time of weaning. The dynamics of infection with *Escherichia*

coli isolated from ordinary mice was not distinguishable from that observed earlier with an enteropathogenic strain of human origin (4). Despite the acute character of the infection thus induced, the growth curve of the contaminated mice did not differ from that of the controls. Nor did any other bacterial culture isolated from ordinary mice prove more active in this regard.[3] In contrast, as already mentioned, a marked depression of the weight curve was observed when newborn SPF mice were contaminated per os with homogenates of the intestines of ordinary mice.

Since the bacterial cultures isolated from the intestinal tract of ordinary mice did not account for the weight-depressing activity of the intestine homogenate, an effort was made to determine whether this activity could be traced to non-bacterial components of the homogenates. To this end, homogenates were clarified by centrifugation, then passed through Millipore filters. When administered per os to newborn SPF mice, such filtrates consistently depressed their growth rates.

The purpose of the following experiments was to separate the weight-depressing agent from the rest of the intestinal flora.

5 adult ordinary mice, approximately 6 wk old, were sacrificed; their intestines were collected, pooled, and homogenized in 25 ml of Tris-buffered salt solution. Half of the homogenate was used without further treatment. The other half was clarified by centrifugation, then filtered through Millipore filters of 0.8 μ porosity. The whole homogenate and the filtrate were used within 1 hr of preparation to contaminate 2-day old NCS mice.

The animals to be contaminated came from 30 litters of NCS mice, reduced to eight newborns per litter (seven in a few cases) 2 days after birth. They were divided into five subgroups of six litters that were contaminated per os as indicated in Table I.

Following contamination, a few of the mothers destroyed their litters within 1 or 2 days. The surviving animals were weighed individually at weekly intervals after weaning.

Table I presents in summary form the numbers of deaths (excluding those that occurred during the first 2 days after contamination) and the weights at 3 wk of age. It shows that administration of the unfiltered homogenate caused a large percentage of deaths, and a marked weight depression in the surviving animals. Filtration of the homogenate considerably reduced its lethal and weight-depressing activity.

Text-fig. 1 illustrates in a schematic manner the results of another experiment in which the growth rate of untreated NCS mice was compared with that of untreated ordinary mice, and of NCS mice contaminated when 2 days old with fecal homogenate obtained from the ordinary mice. In this particular experi-

[3] Recent experiments have revealed that oral contamination of young NCS and COBS mice with *Klebsiella pneumoniae* (type C) causes the death of many animals within 1–2 wk, even when the contaminating dose is extremely small (10^{-5} ml). The course of oral Klebsiella infection and its consequences will be described in a later publication.

ment, administration of the homogenate did not cause any death, but depressed the growth of NCS mice.

Let it be emphasized once more that contamination of newborn NCS mice with fecal material or intestinal homogenate obtained from uncontaminated NCS mice did not produce either obvious signs of disease or weight retardation.

TABLE I

Oral Contamination of Newborn NCS Mice with Intestine Homogenate of Adult Ordinary Mice

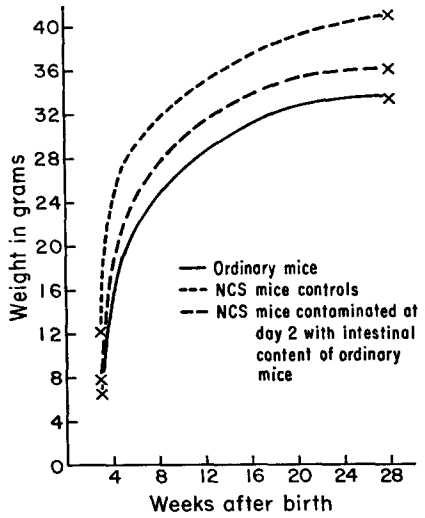
Contaminating dose	Deaths*	Weights at weaning time	
		Range	Average‡
ml per os	%	*g*	*g*
Control (Tris buffer)	0	9.9–14.5	10.8
0.003 homogenate§	100 ‖		—
0.0003 "	30 ‖	6.5–10.9	7.5
0.003 filtrate	12 ‖	7.3–14.7	9.7
0.0003 "	0	8.2–13.8	10.9

* All deaths occurred within 10 days after contamination.

‡ Averages for 35–40 mice.

§Homogenate of stomach and intestine of adult ordinary mice.

‖ Filtrate of intestinal homogenate through Millipore disc of 0.8 μ porosity.



TEXT-FIG. 1. Weight curves of ordinary mice, uncontaminated NCS mice, and NCS mice contaminated at 2 days of age with intestine homogenates of ordinary mice.

The results presented in Table I and Text-fig. 1 are similar to those obtained in an earlier study by allowing newborn NCS mice to be contaminated by contact with SS mice (2). They substantiate the view that the difference in size between NCS and ordinary mice is not genetic in nature but probably has its origin in the indigenous microbiota of these animals.

As was to be expected, contamination of newborn SPF mice (NCS or COBS) with crude homogenates of the intestine of ordinary mice commonly caused paralysis and other neurological disorders, as well as infantile diarrhea. Many animals died shortly after contamination, as seen in Table I. These aspects of the problem will not be considered here. Instead, it will be shown that lasting depression of weight can be achieved by oral contamination of newborn SPF animals with material which does not produce any obvious sign of disease.

Progressive Increase in the Weight-Depressing Activity of Intestinal Content of Newborn SPF Mice after Oral Contamination with Material Obtained from the Intestine of Ordinary Mice.—Experiments were carried out to test the possibility of passing the growth-depressing agent from NCS mouse to NCS mouse by oral contamination with intestinal homogenates from ordinary mice. These experiments revealed that two conditions were necessary to obtain unequivocal results: (*a*) contamination must take place within a few days after birth; (*b*) the intestinal contents must be collected 3–10 days after contamination. These two aspects of the technique will now be discussed briefly.

(*a*) Earlier bacteriological studies have revealed that under normal conditions, the indigenous flora of the mouse gastrointestinal tract does not become fully established until the 12–14th day after birth (5, 6). This probably accounts for the fact that the susceptibility of NCS mice to colonization with *E. coli* (4) and to contamination with other pathogens by exposure to SS mice is extremely high during early life, then decreases sharply as the animal approaches weaning age. In the present study, weight depression of SPF mice could best be achieved by contaminating them with the intestinal contents of ordinary mice within 3 days after birth.

(*b*) The following experiment illustrates that the activity of intestinal filtrates prepared from contaminated NCS mice progressively increases for several days after contamination.

5 ordinary mice were sacrificed at approximately 6 months of age; their stomachs and intestines were pooled and homogenized in 25 ml of Tris-buffered salt solution; the homogenate was centrifuged and filtered through a Millipore disc of 0.45 μ porosity. This filtrate was used to contaminate per os 30 litters of 2 day old NCS mice.

The contaminated NCS mice were sacrificed 1, 2, 3, 6, or 10 days after contamination, the animals from six litters being used for each incubation period. In each case, the stomachs and intestines were homogenized, pooled, and rapidly frozen at −70°C. The activity of these frozen pools was tested by contaminating 2 day old NCS mice per os with homogenates filtered through Millipore discs of 0.45 μ porosity. Other mice were similarly contaminated with the same filtrate diluted 100-fold in Tris-buffered salt solution. In other words, each of

these latter animals received per os less than 0.001 ml of filtrate. Some of the results are summarized in Table II.

As seen in Table II, the filtrates prepared from contaminated mice had little activity 1 and 2 days after contamination. However, activity increased with time, at least until the 10th day.

At the 10^{-2} dilution, the filtrates obtained from animals 6 or 10 days after contamination depressed the weight of NCS mice to which they were administered per os, but did not kill any of them; nor did they cause paralysis, diarrhea, or any obvious symptom of disease. These findings suggested that the growth-depressing activity of the filtrate might be due to the presence of some agent other than the ordinary mouse pathogens.

TABLE II

Comparative Activity of Intestine Filtrates Collected at Various Intervals of Time (1–10 Days) after Contamination of NCS Mice

Filtrates collected after*	Deaths‡ caused by undiluted filtrates	Weight at 3 wk of male NCS mice contaminated* with diluted test filtrates	
		Range	Average§
		g	g
1 day	0/12	10.4–13.1	11.5
2 "	1/12	9.1–11.6	10.0
3 "	6/12	9.1–10.7	9.8
6 "	12/12	8.8–11.5	9.3
10 "	10/12	7.3–11.4	9.7
Control	0/12	11.5–13.6	12.2

* NCS mice were contaminated per os at 2 days of age with test filtrates (Millipore disc 0.45 μ) of intestine homogenates from NCS mice sacrificed 1, 2, 3, 5, or 10 days after oral contamination.

‡ All deaths occurred within 6 days after contamination.

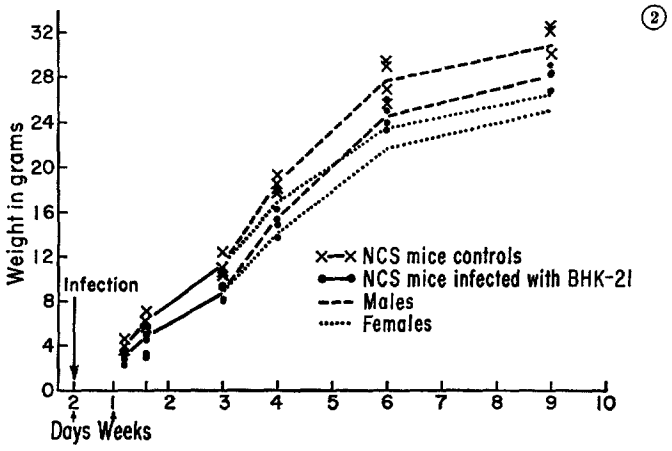
§ Average for approximately 40 animals.

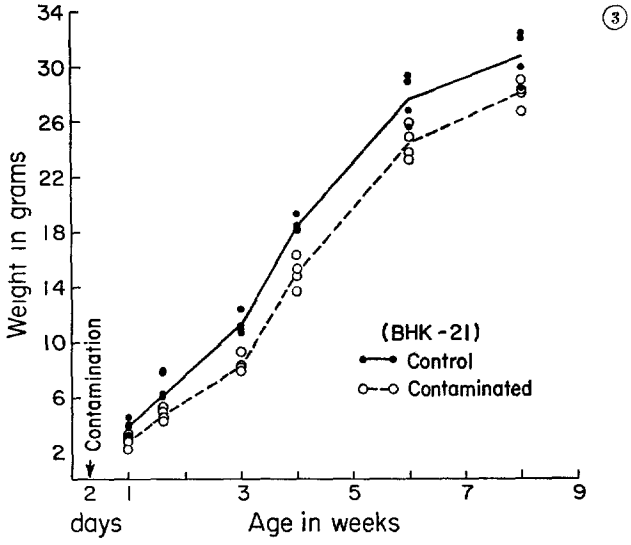
Growth-Depressing Activity of BHK-21 Tissue Cultures Inoculated with Intestinal Filtrates from Contaminated SPF Mice.—Weight depression has been consistently achieved by contaminating 2 day old SPF mice (NCS and COBS) per os with intestinal homogenates filtered through Millipore filters of 0.45 and 0.22 μ porosity. The following experiments show that a weight-depressing agent could be grown in BHK-21 tissue cultures.

NCS mice were contaminated per os 2 days after birth with a 0.45 μ porosity Millipore filtrate of intestinal homogenate prepared from adult ordinary mice. They were sacrificed when 5 wk old at a time when it was obvious that their growth had been stunted. A filtrate was then prepared from homogenates of their intestinal tract. This filtrate was used to inoculate BHK-21 culture as described under Materials and Methods.

After four blind passages, the infected BHK-21 culture showed abnormal growth with long filaments (Fig. 1). Cultures of BHK-21 showing these alterations were used to contaminate 2 day old NCS mice.

Text-fig. 2 and 3 show that oral contamination of 2 day old mice with the





TEXT-FIGS. 2 and 3. Weight curves of NCS mice contaminated orally with fifth and sixth passages, respectively, of infected BHK-21 showing the abnormal growth illustrated in Fig. 1.

Each point represents the average for each box of animals; each curve corresponds to averages for approximately 15–20 males.

agent growing in BHK-21 cultures produced a weight depression similar to that resulting from contamination with intestinal filtrate.[4]

A filtrate (Millipore disc, 0.45 μ) of intestinal homogenate was prepared from NCS mice contaminated with BHK-21 culture (from the experiment represented in Text-fig. 3). This new filtrate was used to contaminate NCS mice 2 days after birth. The filtrate prepared from the intestinal homogenates of these animals was then used to contaminate other newborn NCS mice and this process was continued for two further passages.

Text-fig. 4 *a* and 4 *b* illustrate the growth rates of NCS mice contaminated 2 days after birth with the second mouse passage.

Until the 23rd wk of life, the males from each litter were kept as a group; their growth curves are illustrated in Text-fig. 4 *a*. After the 23rd wk the animals were transferred to stainless cages on wire grids and maintained singly under such conditions thereafter; their weight curves are presented in Text-fig. 4 *b*.

As seen in Text-fig. 4 *b*, both the controls and the contaminated NCS mice lost weight at the time they were transferred from group caging to single caging (23rd wk of life); however, they resumed their growth after becoming adapted to their new surroundings. This phenomenon has been observed on repeated occasions, for example see Text-fig. 8 in the preceding paper (1). Of more relevance to the present study was the finding that the difference between control and contaminated mice persisted throughout the 46 wk of observation. Other experiments now in progress indicate that weight depression can persist for more than 1 yr.

As a result of successive animal passages of the BHK-21 culture by oral contamination of 2 day old NCS mice, the intestinal homogenates of these animals appeared to increase in activity. A filtrate of intestinal homogenate derived from mice that had been contaminated with the fifth mouse passage was titrated by feeding 10-fold serial dilutions (10^{-2}, 10^{-3}, and 10^{-4}) to 2 day old NCS mice (Text-fig. 5).

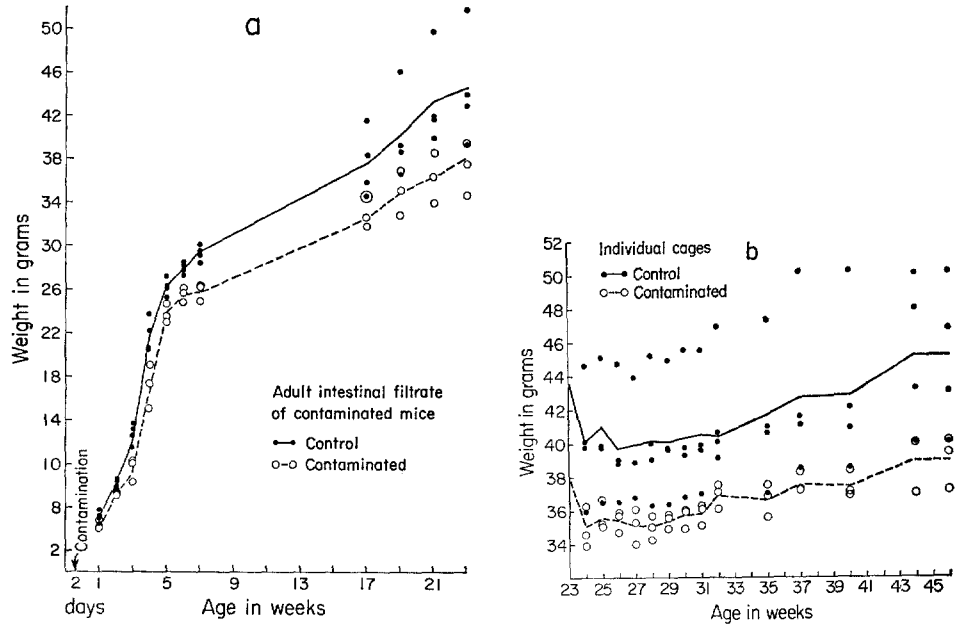
As seen in Text-fig. 5, the weight depression was pronounced even in animals contaminated with the 10^{-4} dilution, which corresponds to a contaminating dose of less than 10^{-5} ml of filtrate.

Growth-Depressing Activity of Primary Mouse Embryo Cell Cultures Inoculated with Filtered Intestinal Homogenate.—In addition to BHK-21, primary mouse embryo cells have provided the only other type of tissue culture in which evidence has been obtained that the growth-depressing agent could multiply in vitro.

[4] In more recent experiments to be reported in detail later, it has been found that depression of growth can be consistently detected by weighing the animals daily after contamination. With active filtrates, differences of weight become obvious 2 days after contamination at 4 days of age, and persist thereafter.

Primary cultures of mouse embryo cells were inoculated with filtered (0.45 μ Millipore disc) intestinal homogenate, prepared from the 10th mouse passage of an active filtrate. The cells were handled as indicated under Materials and Methods. After one blind passage, a few of the tissue cultures exhibited long filaments similar to those seen in infected BHK-21. Unfortunately, it has proved impossible to obtain this type of cell alteration at will in primary mouse embryo cell culture.

Cultures showing the alteration just mentioned were used to contaminate 2 day old NCS mice by the usual technique (Text-fig. 6). In parallel experiments other mice were contaminated with the cell-free medium of infected cultures (Text-fig. 7).



TEXT-FIGS. 4 *a* and 4 *b*. Weight curves of NCS mice contaminated with second mouse passage of infected BHK-21: (*a*) kept in groups of 4–5 mice; (*b*) placed at 23 wk old in single cages.

The points represent the average weights for each box of animals; the curves correspond to approximately 20 males.

The results illustrated in Text-fig. 7 show that the medium in which infected mouse embryo cells had grown produced a weight depression similar to that produced by the infected cells themselves (Text-fig. 6). It appears, therefore, that the active agent is released from the cells into the medium and retains its activity in the intracellular environment.

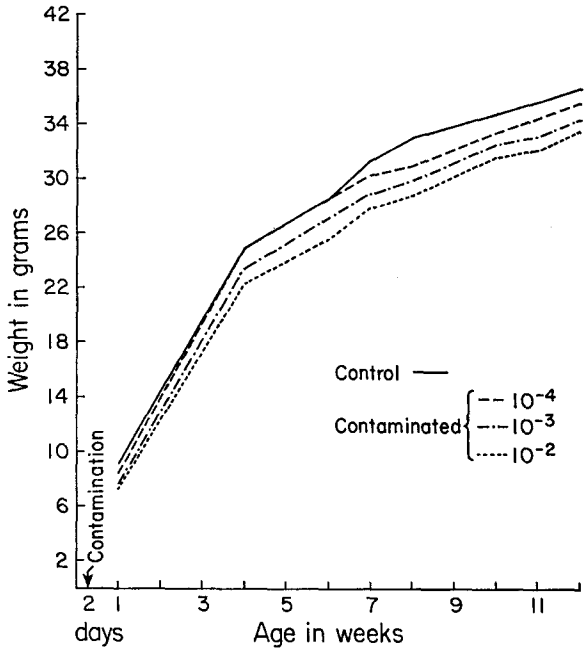
The activity of the material present in the tissue culture was titrated as follows:

10-fold dilutions, in Tris-buffered salt solution, were prepared of a second passage of the agent in mouse embryo cells that had been originally infected with filtered intestinal homog-

enate. These dilutions were administered per os to 2 day old mice. Their weight curves are shown in Text-figs. 8 *a* and 8 *b*.

Text-figs. 8 *a* and 8 *b* show that weight depression occurred in all contaminated animals and that, irrespective of the contaminating dose used, it could be detected 1 wk after contamination and became more pronounced with time.

Examination of the growth curves suggests a curious fact that has been observed in several other experiments. Whereas the largest dose of contaminating



TEXT-FIG. 5. Weight curves of NCS mice contaminated orally with 10-fold dilutions of filtered intestine homogenates.

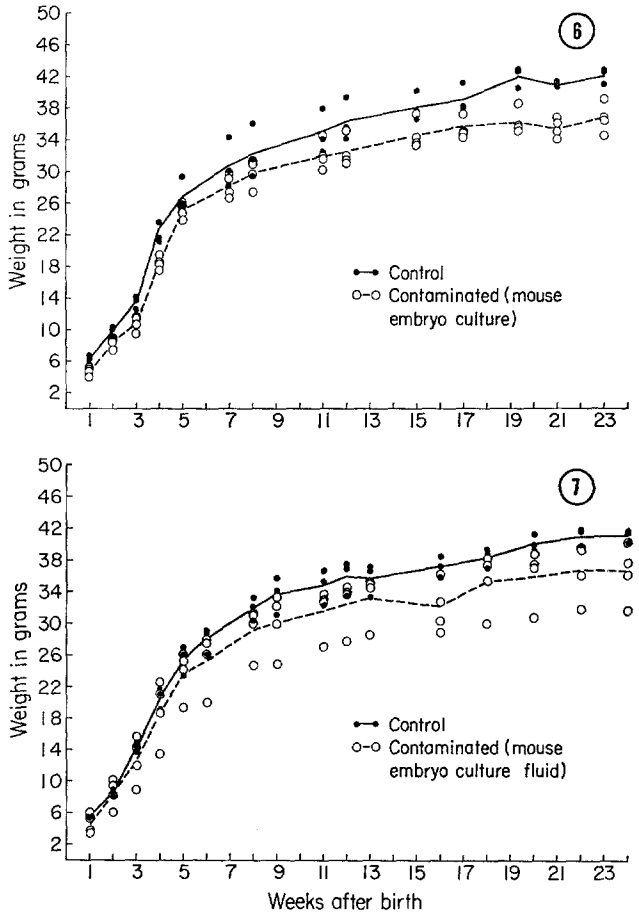
The curves for the first 4 wk correspond to approximately 40 animals (males and females); after 4 wk the curves correspond to males only.

material (undiluted) usually exerts its most pronounced effect immediately after contamination and during the first few weeks, the effect of the smaller dose (10^{-3} dilution) is often weak at first but increases progressively with time. Text-fig. 8 *b* shows that after 12 wk the most profound weight depression occurred in animals receiving the smallest contaminating dose. It seems worth speculating that the animals receiving the undiluted mouse embryo culture rapidly developed a protective immune response against the weight depressing agent, whereas the smallest dose produced a more chronic infection.

The weight depressing activity of the mouse embryo tissue culture was con-

firmed in COBS mice; these were contaminated at 2 days of age with the following materials.

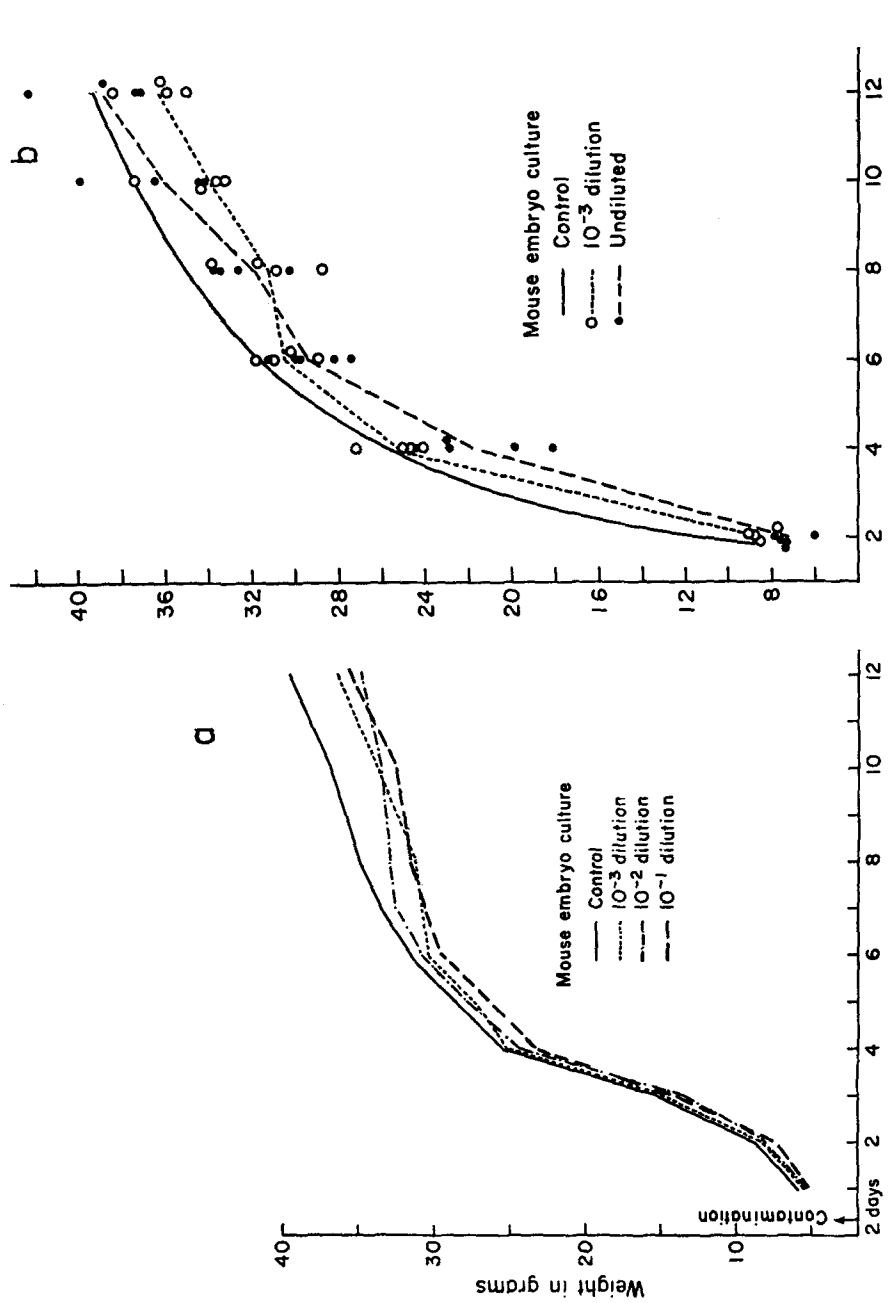
One group of animals received per os a first passage of inoculated mouse embryo culture; a second group received a pool of second and third passage of mouse embryo culture; a third group received a filtrate of intestinal homogenate prepared from contaminated mice showing marked depression of growth.



TEXT-FIGS. 6 and 7. Weight curves of NCS mice contaminated 2 days after birth with mouse embryo infected cells (Text-fig. 6); or with cell-free medium of infected mouse embryo cultures (Text-fig. 7).

On both graphs each point represents the average weight for each box of animals; each curve corresponds to approximately 15–20 males.

As seen in Table III the second and third passage in mouse embryo cell cultures proved at least as active as the first passage; in all three groups, depression of growth could be recognized within 1 wk after contamination.



TEXT-FIGS. 8 *a* and 8 *b*. Weight curves of NCS mice contaminated 2 days after birth with 10-fold dilutions of the second passage of mouse embryo cells infected with filtered intestine homogenates. Text-fig. 8 *b* presents in greater detail the comparative weight gains of mice contaminated with either undiluted infected cells (dots) or 10^{-3} dilution thereof (circles). Note that weights for the controls are presented as a smooth curve approximating the actual values given in Text-fig. 8 *a*.

Transmission of the Growth-Depressing Effect from One Generation to the Next.—As mentioned earlier, the growth-depressing agent is widely distributed among three of the ordinary mouse colonies that we have tested. The following experiment shows that it can also be transmitted from one generation to the next among SPF mice.

Adult NCS males and females were contaminated when 2 days old with filtered intestinal homogenates derived from mice which had been contaminated with a 10^{-2} dilution of active intestinal filtrate. The animals were mated at 8 wk of age, one couple per cage. Noncontaminated NCS controls of the same age were mated at the same time, under the same conditions. In this experiment, the litters were not reduced as usual to 8, but kept at 9–10 per cage. The weights of the progeny of the two groups were recorded at weekly intervals, as illustrated in Text fig. 9.

TABLE III

Weight Depression in COBS Mice Contaminated with Infected Mouse Embryo Cells or with Filtrate of Homogenized Intestines

Contaminating Material	Weight at indicated age			
	1 wk		3 wk	
	Range	Average*	Range	Average*
	g	g	g	g
Control	6.0–7.1	6.3	16.2–18.7	17.5
Mouse embryo cells‡	5.6–4.9	5.1	15.7–18.8	16.9
Mouse embryo cells§	5.3–4.8	5.0	13.3–16.8	15.0
Intestinal filtrate‖	3.0–4.4	3.7	12.5–15.9	14.6

* Average for approximately 30 mice per group.

‡ First passage of mouse embryo tissue culture infected with bacteria-free filtrates of homogenized intestines from contaminated NCS mice.

§ Pool of second and third passage of mouse embryo cells infected as above.

‖ Filtrate of homogenized intestines from stunted NCS mice.

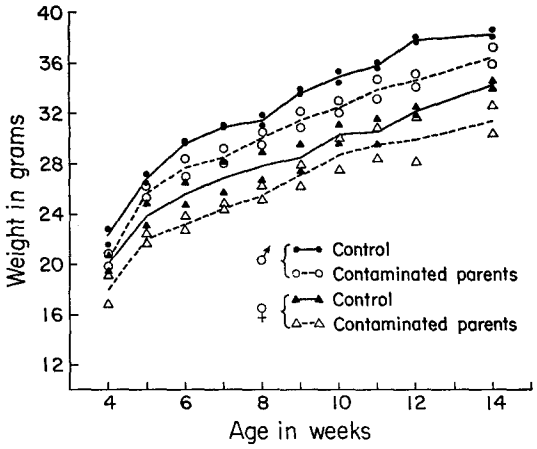
NCS mice that had been contaminated at birth were as fertile as uncontaminated controls. Furthermore, contamination did not increase the incidence of infantile mortality in their progeny. However, animals born from contaminated NCS mice were generally smaller at weaning time and remained smaller thereafter than the progeny of control NCS mice (Text-fig. 9). In other words, the weight-depressing agent can be transmitted from one generation to the next, without causing any obvious disease except a reduction in size.

Preliminary Observations on the Characteristics of the Growth-Depressing Agent.—In many experiments over the past 3 yr, intestinal homogenates that had been shown to be capable of depressing the weight of SPF mice were filtered through Millipore discs of 0.8, 0.45, 0.22, and 0.10 μ porosity. When the filtrates

were administered per os to either NCS or COBS mice, 2 days old, weight depression was achieved with all of these preparations, except with the ones passed through the 0.10 μ discs. These findings suggest that the particle size of the agent is of the order of 0.1 μ.

The agent is partially or completely inactivated by (*a*) 3 hr exposure to pH 3.0 at room temperature; (*b*) 1 hr heating at 50°C in Tris-buffered salt solution; and (*c*) contact with ether at 4°C for 16 hr.

Histological studies have not yet been made of the tissues of animals contaminated with the filterable agent. However, many of these animals, contaminated when 2 days old and exhibiting marked weight depression, were sacrificed and



TEXT-FIG. 9. Weight curves of NCS mice born from uncontaminated and contaminated parents. Each point represents the average weight for each box of animals; each curve corresponds to approximately 20 animals.

autopsied at different periods of time after they had reached adult age. No macroscopic abnormality could be detected under the dissecting microscope in any of the organs.

The metabolic disturbances caused by contamination and resulting in weight depression are being investigated by our colleague Dr. Chi-Jen Lee.

SUMMARY

Certain specific-pathogen-free (SPF) mice bred and maintained under semi-protected conditions have an intestinal flora which is qualitatively simpler (although not quantitatively smaller) than that of mice of the same genetic stock produced under ordinary conditions. They are also heavier at weaning time, grow at a faster rate, and reach a greater adult weight than ordinary mice.

When SPF mice are contaminated per os shortly after birth with certain bacterial cultures isolated from the intestinal contents of adult ordinary mice, these bacteria multiply extensively throughout the gastrointestinal tract and persist at extremely high levels until weaning time. Such bacterial infections do not affect significantly either weaning weight, growth rate, or maximum adult weight.

In contrast, weight depression could be consistently brought about by contaminating newborn SPF mice per os with bacteria-free filtrates of homogenates of intestines from ordinary mice.

The weight-depressing agent passed through Millipore discs of 0.45 and 0.22 μ porosity, but was held back at 0.10 μ porosity.

The depression of weight caused by either intestine homogenate or filtrates thereof could be detected within a few days after contamination (of 2 day old mice) and persisted throughout the adult life of the contaminated animals. When intestine homogenate of the SPF mice used in this study were introduced per os into newborn SPF mice, they did not affect their growth rate or adult weight.

On several occasions, but not consistently, bacteria-free filtrates capable of depressing the weight curve of SPF mice produced alterations in the appearance of tissue cultures of BHK-21 and mouse embryo cells. When tissue cultures so infected were introduced into newborn SPF mice, the weight of these animals was depressed early and lastingly.

An agent exhibiting weight-depressing activity has been transferred from mouse to mouse over many passages by contaminating newborn SPF animals per os.

Weight depression was achieved with extremely small doses of material (10^{-5} ml of intestine homogenate of 10^{-4} of mouse embryo culture). Under these conditions, none of the animals showed obvious signs of disease except reduced weight.

Only very young SPF mice (preferably less than 3 days old) proved susceptible to the weight-depressing effect of the filtrates of intestine homogenates or of infected tissue cultures prepared therefrom. After oral contamination, it took approximately 1 wk before the intestinal homogenate obtained from contaminated animals exhibited a high level of weight-depressing activity.

The growth-depressing effect could be transmitted from one generation to the next by mating SPF mice that had been contaminated shortly after birth and that were consequently smaller than control SPF animals.

BIBLIOGRAPHY

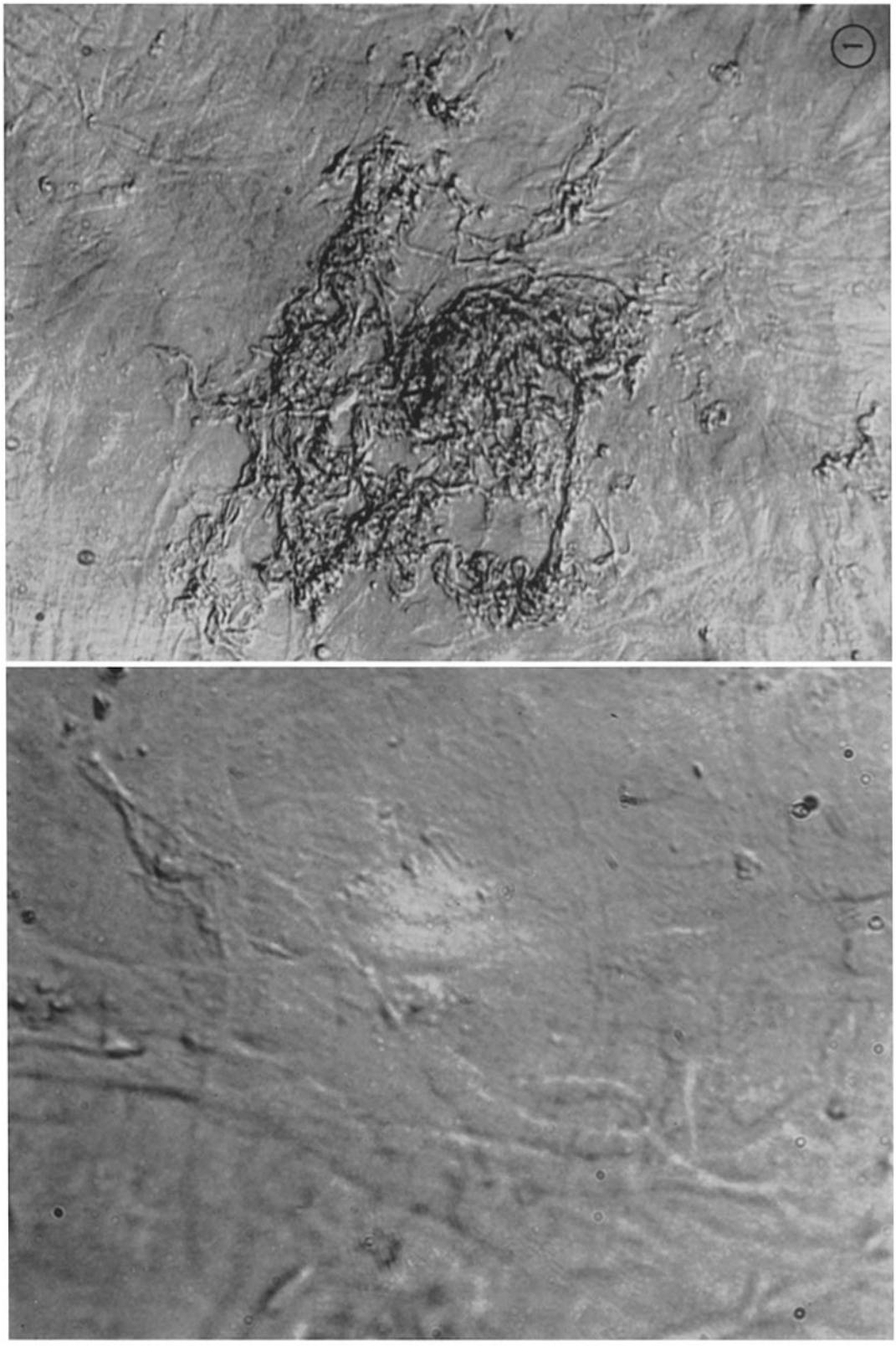
1. Dubos, R., R. W. Schaedler, and R. Costello. 1968. Lasting biological effects of early environmental influences. I. Conditioning of adult size by prenatal and postnatal nutrition. *J. Exptl. Med.* **127**:783.

2. Dubos, R., and R. W. Schaedler. 1960. The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. *J. Exptl. Med.* **111**:407.
3. Nelson, J. B., and G. R. Collins. 1961. The establishment and maintenance of a specific pathogen-free colony of Swiss mice. *Proc. Animal Care Panel.* **11**:65.
4. Mushin, Rose, and René Dubos. 1965. Colonization of the mouse intestine with *Escherichia coli*. *J. Exptl. Med.* **122**:745.
5. Schaedler, R. W., R. Dubos, and R. Costello. 1965. The development of the bacterial flora in the gastrointestinal tract of mice. *J. Exptl. Med.* **122**:59. Dubos, R., R. W. Schaedler, R. Costello, and P. Hoet. 1965. Indigenous, normal, and autochthonous flora of the gastrointestinal tract. *J. Exptl. Med.* **122**:67. Dubos, R., and R. W. Schaedler. 1964. The digestive tract as an ecosystem. *Am. J. Med. Sci.* **248**:267.
6. Savage, D., R. Dubos, and R. W. Schaedler. 1968. The gastrointestinal epithelium and its autochthonous bacterial flora. *J. Exptl. Med.* **127**:67.

EXPLANATION OF PLATES

PLATE 90

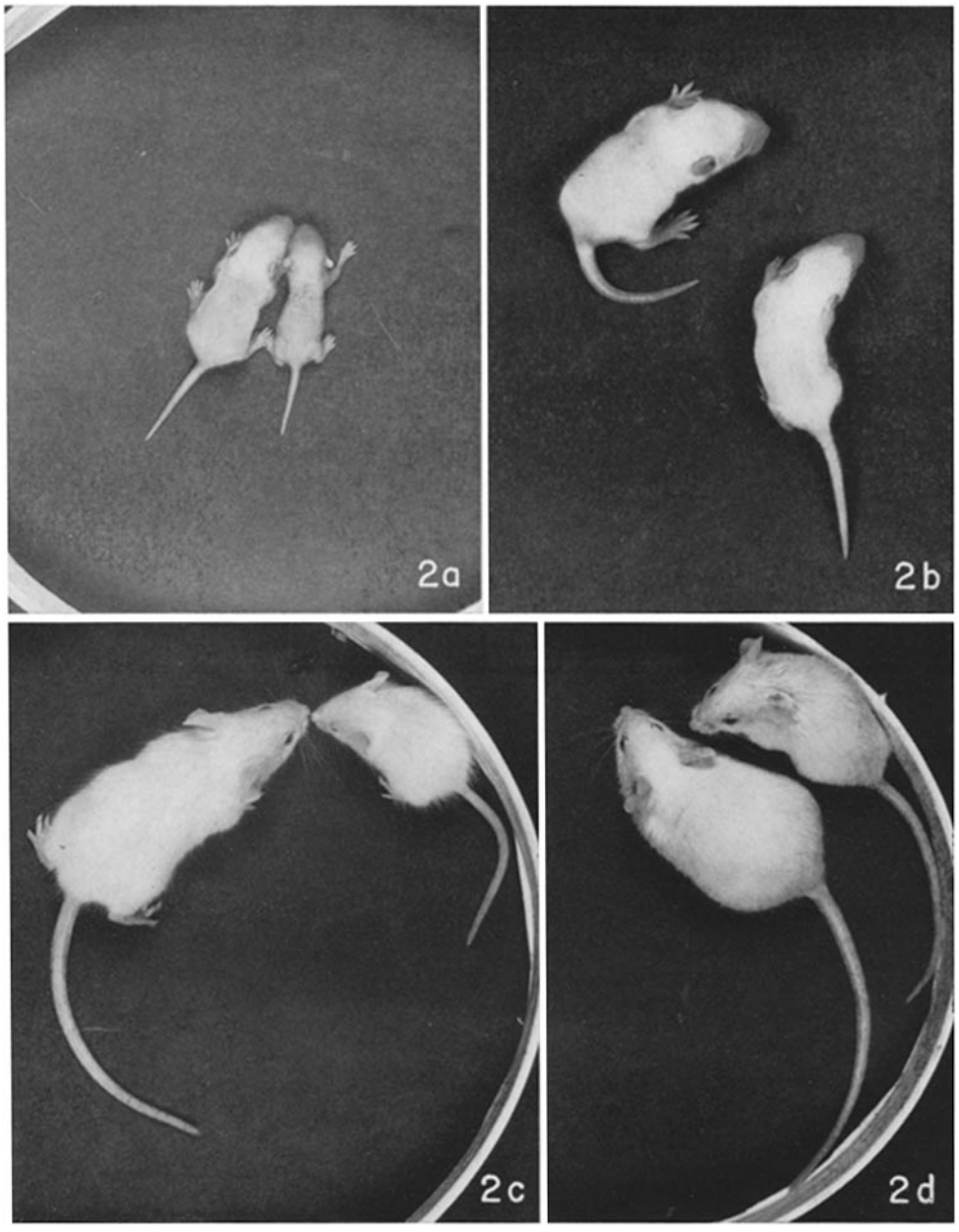
FIG 1. BKH-21 cells. Left, controls. ×400. Right, cells infected with filtered intestine homogenates. ×60. The abnormal growth became detectable at the fifth passage of infected cells.



(Seravalli and Dubos: Neonatal contamination and weight depression)

PLATE 91

FIG. 2. NSC mice contaminated when 2 days old with material grown on mouse embryo tissue culture: (*a*) 4 days after contamination; (*b*) 7 days after contamination; (*c*) 3 wk after contamination; (*d*) 5 wk after contamination.



(Seravalli and Dubos: Neonatal contamination and weight depression)