THE APPEARANCE OF A HEPATOTROPHIC VIRUS IN MICE THYMECTOMIZED AT BIRTH

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Mice thymectomized within strict time-limits at birth develop a wasting syndrome and die prematurely before they are 2 to 3 months old (1, 2). Because their immune response to skin homografts (3, 4) and to grafts of foreign cells (2, 5) is known to be impaired it is generally assumed that all their immunological defences are diminished and that they die from intercurrent infection. However, contrary to expectation, it was found that neonatally thymectomized wasting mice could, in certain circumstances, synthesize γ-globulin and circulating antibody just as effectively as normal animals (5), and we were also unable, in preliminary experiments, to isolate any specific bacterial or viral agents from their organs or blood (5). Nevertheless, it still seemed unwise to rule out the complication of an incidental infection in such debilitated animals and this possibility was investigated further.

The wasting symptoms that follow neonatal thymectomy include a gradual deterioration in physical condition, progressive loss of weight, a curious high-stepping but coordinated gait, and a very severe depletion of lymphocytes in the lymph nodes, spleen, and peripheral blood. The immunological significance of the lymphocyte depletion has already been discussed extensively (2, 5, 6) but another prominent pathological change, the occurrence of macroscopic lesions in the liver, has received very little attention. Because this feature seemed to be most consistent with the presence of a transmissible agent, the liver and spleen of thymectomized wasting mice were passaged, either as tissue suspensions or cell-free extracts, to newborn recipients and the results are reported below.

Materials and Methods

Animals.—Two inbred strains (C57BL; C3H/Bi), 1 F1μhybrid (C57BL × C3H/Bi), and 1 outbred stock (TO) of mice were used. With one exception, all the donors were thymectomized within 24 hours of birth using anaesthesia produced by cooling (7). Wasting donors were killed in the final stage of debilitation and so varied as to sex, age, and extent of

liver damage. Recipients were injected when newly born (1 to 3 days) or at weaning (19 to 21 days) and were usually intact, although a small number of thymectomized babies were included in some of the experiments. The completeness of all thymectomies was checked at postmortem and also by histological examination.

Tissue Suspensions.—Tissues were removed from donors under sterile conditions, weighed, and dissociated in sterile isotonic saline by squeezing through a disposable syringe. After dilution, the tissue slurry was injected intraperitoneally into the recipients in a volume of 0.05 to 0.1 ml. Unless otherwise stated, donor tissues were not pooled and a standard dose equivalent to 30 mg liver or 3 to 4 mg spleen was given to each recipient.

Cell-Free Extracts.—Livers were ground in Hanks' solution with sterile sand and the suspension centrifuged for 15 minutes at 1500 *g*. The supernatant was then filtered under pressure through a bacterium-tight sintered glass filter (porosity 5). Extracts were used immediately and all recipients were given 0.05 to 0.1 ml extract intraperitoneally.

Histology.—Liver, spleen, inguinal lymph nodes, gut and thymus (where available), and other selected organs were fixed in 10 per cent neutral formol saline for staining with Ehrlich's haematoxylin and eosin. Brain sections were cut after decalcifying the head in versene and vacuum embedding.

RESULTS

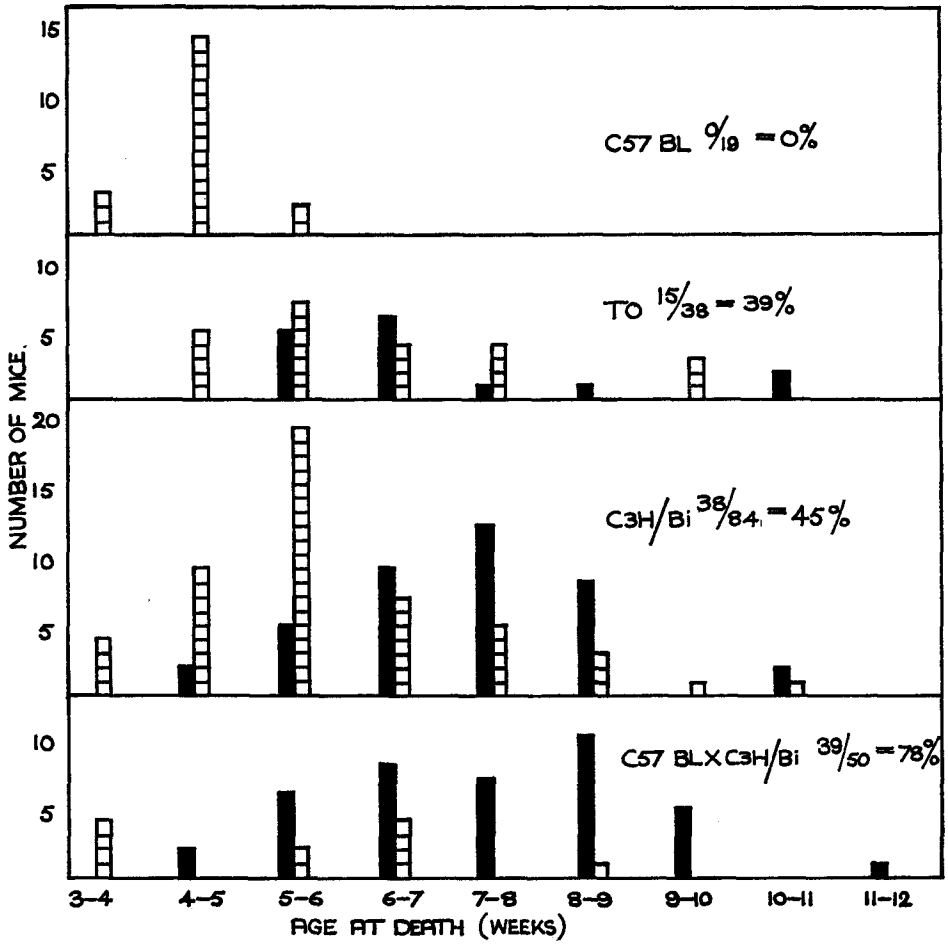
Incidence of Liver Lesions.—The incidence of liver lesions in 191 thymectomized wasting C3H/Bi, C57BL, F1 (C57BL × C3H/Bi), and TO mice was calculated from postmortem data collected over a period of 18 months. The records referred to animals which had died after wasting or were killed when severely debilitated. Macroscopic lesions were easily detected but the livers of all the mice were also examined microscopically.

Text-fig. 1 shows that, although liver lesions were a prominent feature of the wasting syndrome, they did not appear in all thymectomized wasting mice. For instance, none were found in the C57BL strain although 45 per cent of the C3H/Bi mice were affected, and the incidence increased to 78 per cent in F1 hybrids of the 2 strains (C57BL × C3H/Bi). Lesions were seen in 39 per cent of the outbred stock. There was a tendency for the lesions to occur in animals dying comparatively late and we therefore assumed that their absence in the C57BL strain was due to the fact that these mice waste precipitately and die even earlier than those of other strains (2, 5). In case the incidence figures had been influenced by the fact that a number of the wasting mice had been killed rather than allowed to die they were recalculated on the basis of mode of death. However, it was found that the incidence of liver lesions in each strain remained the same whether the mice had died or had been killed when judged to be *in extremis*.

Thus, thymectomized mice inevitably waste and die whether they show liver lesions or not and the incidence of the lesions clearly varies with the strain of mice used and probably also with the age at death. These facts implied that, if the liver lesions were caused by a transmissible agent, this agent was not the cause of death but was, rather, of an incidental nature. In order to test this possibility, tissues for passage were taken from thymectomized wasting

mice with or without liver lesions and from thymectomized but healthy donors killed before the onset of wasting.

Passage of Necrotic Liver from Thymectomized Wasting Donors.—Suspensions of necrotic liver taken from 4 thymectomized wasting mice of various strains



TEXT-FIG. 1. Incidence of liver lesions in mice thymectomized within 24 hours of birth. □ wasting mice without liver lesions; ■ wasting mice with liver lesions.

were given intraperitoneally to 52 intact baby recipients. All died with severe liver damage 3 to 7 days after injection and within 24 hours of the first white focal lesions being seen through their paper-thin skin (Table I).

Serial passage of liver tissue was then attempted using 1 C3H/Bi and 1 hybrid mouse as original donors. Both these animals were thymectomized

within 24 hours of birth, both were wasting, and both had liver lesions when they were killed at 50 and 51 days of age respectively. Liver from each donor was passed serially through intact newborn C3H/Bi recipients for 5 consecutive passages. Two or 3 babies from each passage were killed to provide a pool of material for the next passage and all recipients were given the standard dose

TABLE I

Passage of Necrotic Liver from Thymectomized Wasting Donors in Intact Newborn Recipients

Donor strain	Recipient strain	No. mice with lesions / No. mice injected	Mean survival time and range
			days
TO	TO	13/13	4.8 (4 to 5)
C57BL × C3H/Bi	TO	5/5	5.0
	C3H/Bi	6/6	6.2 (4 to 7)
C57BL × C3H/Bi + TO (pool)	TO	28/28	3.7 (3 to 4)

TABLE II

Serial Passage of Necrotic Liver from Thymectomized Wasting Donors in Intact Newborn C3H/Bi Recipients

Serial passage A			Serial passage B		
Donor, male C3H/Bi aged 50 days			Donor, female C57BL × C3H/Bi aged 51 days		
Pass No.	No. mice with lesions / No. mice injected	Mean survival time and range	Pass No.	No. mice with lesions / No. mice injected	Mean survival time and range
		days			*days*
1	8/9	7.9 (6 to 12)	1	11/11	5.8 (3 to 7)
2	3/3	4.7 (3 to 8)	2	6/6	8.0 (5 to 14)
3	5/5	8.4 (5 to 12)	3	4/6	8.5 (7 to 11)
4	9/11	6.6 (5 to 9)	4	11/11	8.3 (6 to 11)
5	8/8	5.5 (5 to 7)	5	9/9	5.4 (5 to 7)

equivalent to 30 mg liver tissue. In the course of the 2 serial passages 33/36 (passage A) and 41/43 (passage B) of the recipients died with fulminating hepatitis 3 to 14 days after injection (Table II). Both males and females were affected and genetic disparity between the original donor and the subsequent recipients did not impede passage. The virulence of the liver agent was already maximal at the first pass and the mean age of death did not alter with successive passages. The 5 survivors were entirely normal when killed 100 to 124 days later and none of the control litter mates left uninjected at each stage of the 2

passages died or showed any overt signs of illness although they had been in close contact with the treated animals.

These results demonstrated that a transmissible agent was present in the necrotic livers of thymectomized wasting mice which produced similar but much more severe liver damage when passaged in newborn recipients.

Passage of Spleen from Thymectomized Wasting Donors.—Although macroscopic lesions are usually seen only in the livers of thymectomized wasting mice other organs were also tested for the presence of the hepatotrophic agent. Suspensions of the spleen as well as of the necrotic liver of a thymectomized

TABLE III

Passage of Spleen from Thymectomized Wasting Donors with Liver Lesions in Intact and Thymectomized Newborn Recipients

Donor strain	Recipient strain	Tissue passaged	No. mice with lesions / No. intact mice injected	No. mice with lesions / No. thymectomized mice injected	Mean survival time and range	
					Intact	Thymectomized
					days	*days*
TO	TO	Liver	8/8	5/5	4.8 (4 to 5)	5.0
		Spleen	7/7	3/3	4.4 (4 to 5)	4.7 (4 to 5)
C3H/Bi	TO	Spleen	9/9	—	4.0	—
C57BL × C3H/Bi	C57BL × C3H/Bi	Spleen	9/9	—	9.0	—

wasting TO donor aged 58 days were inoculated separately into 3 litters of 1-day-old TO mice. These litters included babies which were intact or had been thymectomized on the day of birth. In 2 additional experiments only intact recipients were used and the donors were a F1 hybrid thymectomized 60 hours after birth which wasted at the late age of 106 days and an extremely debilitated C3H/Bi mouse killed at 60 days; both had liver lesions.

Both the spleen and the liver suspensions produced severe and lethal liver damage in the thymectomized and in the intact recipients and in the same period of time. The efficacy of spleen preparations was also confirmed in the subsequent experiments (Table III).

Passage of Macroscopically Normal Liver from Thymectomized Wasting Donors.—Macroscopically normal livers from 4 thymectomized wasting donors were sampled for histological examination and suspensions then passaged individually into groups of intact newborn mice. The liver of 1 donor killed

5/5 recipients within 6 to 10 days of injection and was found subsequently to have microscopic lesions. However, the livers of the remaining 3 donors that did not produce liver necroses in their respective recipients were quite normal when examined histologically (Table IV).

Two facts were now obvious, namely, that the agent could only be passaged readily from thymectomized donors which had liver lesions and that the lesions, when they did occur, were found only in wasting animals. It seemed likely therefore that the agent would not be transmitted from healthy thymectomized mice killed prior to the onset of wasting and this supposition was tested in the following experiment.

Passage of Liver from Thymectomized Donors Killed Prior to Wasting.—Liver suspensions prepared from C3H/Bi mice thymectomized at birth and

TABLE IV

Passage of Macroscopically Normal Liver from Thymectomized Wasting Donors in Intact Newborn Recipients

Donor strain	Recipient strain	No. mice with lesions / No. mice injected	Mean survival time and range	Histological appearance of liver
			days	
TO	TO	0/16	—	Normal
TO	TO	0/9	—	Normal
C57BL × C3H/Bi	C57BL × C3H/Bi	0/9	—	Normal
C3H/Bi	C3H/Bi	5/5	7.4 (6 to 10)	Necrotic

killed while still healthy at 7, 14, 21, and 28 days of age were passed to 4 groups of intact C3H/Bi babies. None of the 29 recipients died or showed any evidence of liver damage when killed 85 to 101 days later. Liver suspensions from intact donors of comparable age were also completely ineffective (Table V).

Although these results confirmed the previous prediction they also revealed a flaw inherent in all the experiments. Because the only criterion of the presence of the liver agent was its transmissibility it was impossible, in a negative experiment, to decide whether the agent was completely absent or only present in very low concentrations. The importance of this fact is emphasized later in the general discussion.

Infectivity of the Liver Agent.—The marked difference between the severity of the liver damage produced in the intact newborn recipients and the relatively slight focal necrosis seen in the majority of the thymectomized donors prompted a further enquiry into the relationship between age and susceptibility.

The necrotic livers from 2 thymectomized and wasting TO donors were pooled and tested in both intact newborn and weanling TO mice. Although all the babies died with liver lesions 4 to 9 days after injection the weanlings

remained quite healthy and had histologically normal livers when killed 10 weeks later (Table VI). No lesions were seen in another series of weanlings killed only 6 days after injection but we cannot exclude the possibility that the older animals suffered a transient low-grade infection particularly since no attempt was made to relate the dose used to body weight.

TABLE V

Passage of Liver from Thymectomized Healthy C3H/Bi Donors in Intact Newborn C3H/Bi Recipients

Age of donor	No. of donors	No. mice with lesions / No. mice injected	No. mice with lesions / No. control mice*
days			
Donors	Thymectomized		
7	2	0/5	0/4
14	1	0/8	0/4
21	1	0/4	0/3
28	2	0/12	0/7
Donors	Intact		
7	2	0/4	—
14	2	0/8	0/5
21	2	0/12	0/5
28	1	0/8	0/4

* Uninjected litter mates.

TABLE VI

Passage of Necrotic Liver from Thymectomized Wasting TO Donors in Intact Newborn and Weanling TO Recipients

Age of recipient	No. mice with lesions / No. mice injected	Mean survival time and range
days		*days*
1	12/12	6.0 (4 to 9)
21	0/19	—

Physical Properties of the Liver Agent.—The liver agent was resistant to cold but was inactivated by heat.

Necrotic livers, taken from 3 thymectomized wasting donors and stored whole or homogenized in 6 per cent glucose at −70°C for 23, 52, and 211 days respectively, produced lethal liver damage in intact new born recipients within 5 to 10 days of injection. In another experiment 11 newborn TO mice also died with liver lesions 5 to 10 days after receiving a suspension of liver that had been stored whole for 214 days, but their litter mates survived the injection of

the same material that had been heated in a water bath for 1 hour at 57°C (Table VII).

Transmission by Cell-Free Liver Extracts.—A cell-free extract was prepared in Hanks' solution from the pooled livers of 2 C3H/Bi donors thymectomized at birth and killed 43 days later with wasting and liver lesions. The extract was injected immediately into intact babies of the same strain randomly

TABLE VII

Inactivation of Necrotic Liver from a Thymectomized Wasting TO Donor as Tested in Intact Newborn TO Recipients

Treatment	No. mice with lesions / No. mice injected	Mean survival time and range
		days
Untreated liver suspension	11/11	7.0 (5 to 10)
Heat-inactivated* liver suspension	0/11	—

* Liver suspension heated in a water bath for 1 hour at 57°C.

TABLE VIII

Transmission of a Cell-Free Extract Prepared from the Necrotic Liver of Thymectomized Wasting TO Donors

Recipient	Treatment	No. mice with lesions / No. mice injected	Mean survival time and range
			days
Intact newborn C3H/Bi	Cell-free extract	8/8	5.5 (4 to 7)
	Crude residue	5/7	4.8 (4 to 7)
Thymectomized newborn TO	Cell-free extract	4/4*	5.0

* Pooled liver suspension from 2 recipients passed to newborn recipients; see Table IX.

selected from 4 litters and their litter mates were given the same volume (0.05 ml) of the crude tissue residue. The cell-free extract produced lethal liver lesions in all 8 recipients within 7 days and the crude suspension killed 5/7 of their litter mates in the same time (Table VIII).

The cell-free extract was also given to 1-day-old TO babies which had been thymectomized within 12 hours of birth and they too died with liver lesions 5 days after injection. A pooled liver suspension from 2 of these thymectomized recipients was then passed routinely into intact TO babies but in this experiment doses equivalent to 20 mg, 2.0 mg, and 0.2 mg liver tissue per mouse were given. All the treated mice died with liver lesions within 4 days but their untreated litter mates survived (Table IX).

Pathology.—The descriptive pathology which follows includes details of the histological changes seen in thymectomized wasting donor mice with macroscopic liver lesions and those seen in newborn recipients of tissue suspensions prepared from both fresh and stored material and in recipients of cell-free liver extracts.

Pathology of Thymectomized Wasting Donors with Macroscopic Liver Lesions.—It should be emphasized that lymphoid depletion and replacement with reticular tissue occur in the spleen, lymph nodes, and Peyer's patches of all thymectomized mice whether they show liver lesions or not. However, this striking and characteristic picture is not described in this section which is concerned only with those changes specifically indicating the presence of a hepatotrophic virus.

TABLE IX

Passage of Necrotic Liver from Newborn Thymectomized TO Mice Given Cell-Free Liver Extract (as Tested in Intact Newborn TO Recipients)

Dose liver, mg/mouse	No. mice with lesions / No. mice injected	Mean survival time and range
		days
20.0	6/6	3.7 (3 to 4)
—	0/5	—
2.0	5/5	3.0
—	0/4	—
0.2	4/4	3.0
—	0/3	—

Liver: At autopsy the liver, which is usually of normal size, may be dotted with white or yellow foci (Fig. 1) or covered with irregular white patches of confluent lesions or be uniformly pale brown in colour with a spongy, pitted, surface. These changes indicate a progression from mild infection to fulminating hepatitis but the latter occurs relatively infrequently and, in the majority of animals, liver damage is only slight to moderate. A greyish exudate sometimes covers the abdominal surface of the diaphragm and the liver capsule.

Histologically, the areas of necrosis contained eosinophilic hyaline masses and aggregates of hyperchromatic nuclear debris. Intracytoplasmic basophilic rods and granules and multinucleate giant cells were also present. Collections of polymorphonuclear leucocytes were frequently seen and histiocytic infiltration also occurred (Figs. 4 *a* and 4 *b*). The cellular infiltration extended into the walls of veins which also showed deposits of pyknotic debris in their lumina. The cytoplasm of the hepatic cells adjacent to the necrotic areas sometimes showed fatty change but in only one case was this extensive. The serosal cells

covering the capsule were swollen and spherical and often coated with fibrinous exudate.

Spleen and lymph nodes: By comparison, the spleen and lymph nodes were little affected and contained only scattered aggregates of pyknotic cells.

Other organs: Similar accumulations of inflammatory cells and hyperchromatic nuclear debris were also found in the pericardium, brain, meninges, and lungs. There was some evidence of inflammation in the other serous membranes, particularly the peritoneum, which were focally infiltrated with polymorphs and occasional multinucleate giant cells.

The pathogenic action of all tested strains of mouse hepatitis virus is enhanced by the murine blood parasite *Eperythrozoön coccoides* and in the case of the virus known as MHV-1 a fatal disease is produced in weanling mice (8, 9). We are grateful to Dr. A. W. Gledhill and Dr. Janet S. F. Niven for establishing a similar relationship to *E. coccoides* infection with suspensions of necrotic livers from 3 thymectomized wasting animals, death occurring in 5 to 7 days. Although in weanlings not infected with *E. coccoides* small liver lesions were seen only irregularly, in all instances typical lesions were found on histological examination. In tissues from thymectomized wasting mice which showed liver lesions Dr. Niven stated that the histological appearances were compatible with the presence of a mouse hepatitis virus, probably MHV-1, as described by Gledhill, Dick, and Niven (10). It should be noted in this context that we have not detected *E. coccoides* in blood smears taken from thymectomized wasting C3H/Bi, C57BL, hybrid C57BL × C3H/Bi, or TO mice.

Pathology of Intact Newborn Recipients.—The effect of injecting necrotic tissues or cell-free liver extracts into intact baby mice was both rapid and fatal and the same sequential liver changes described for the thymectomized wasting donors could be seen proceeding in the recipients (Figs. 2 and 3). However, the majority of the recipients showed infinitely more extensive and extremely severe liver involvement as compared with the thymectomized wasting donors and the first sporadic white foci appeared and spread to cover the whole organ in a matter of hours. Fresh blood was often found in the intestines at autopsy and the lungs were pale with dark red patches. Although the spleen was slightly enlarged it was usually uniform in colour and consistency but the thymus was small and discoloured.

Liver: Histologically the liver lesions were very similar to, but more extensive than, those seen in the thymectomized donors although there was less infiltration of polymorphs and histiocytes and fewer multinucleate giant cells were found (Fig. 5). Megakaryocytes and foci of erythropoiesis were present but were also noted in untreated babies.

Thymus: Apart from the liver, the thymus also showed consistent and severe damage and the normal architecture of the cortex was virtually destroyed. It is not yet known whether this destruction is caused specifically by the virus or is

a non-specific stress reaction. Large accumulations of pyknotic nuclear debris were common, polymorphs were often present and, in one case, a multinucleate giant cell was seen (Fig. 6).

Spleen: The spleen, as in the thymectomized donors, was relatively unaffected although there were sometimes excessive numbers of polymorphs in its sinuses and some deposits of intravascular debris. Microabscesses and slight evidence of serositis were also encountered.

Other organs: There was little pathological change in the other organs although small accumulations of nuclear debris, seen sporadically in the lymph nodes (Fig. 7) and lungs, may have been embolic in origin. The lungs regularly showed areas of collapse and there was slight myeloid hyperplasia in the marrow of the cranial bones. Although populations of *Eimeria falciformis* were found in the small bowel of both treated and untreated babies they appeared to be somewhat greater in the treated animals.

Thus, the granulomatous foci seen in the livers of some thymectomized wasting donor mice are due to an hepatotrophic virus, probably MHV-1. The virus produces a similar but fatal hepatitis in intact newborn recipients and can be transmitted with equal facility by suspensions of liver or spleen and by cell-free liver extracts.

DISCUSSION

It has been shown that the agent responsible for the liver lesions which appear in a proportion of thymectomized wasting mice is a hepatotrophic virus very similar to MHV-1. The virus produces a fatal hepatitis when inoculated into intact newborn mice but is much less pathogenic for intact weanlings unless potentiated by the blood parasite *Eperythrozoön coccoides.*

Of the other known hepatotrophic viruses, some have arisen spontaneously (11, 12), but more usually they have been detected during experiments involving the induction of tumours by cell-free extracts or the transplantation of neoplasms (13–16). Even so, the virus rarely explodes into epidemic activity although Rowe, Hartley, and Capps (17) have recently described a spontaneous hepatotrophic virus said to produce a highly contagious, prevalent, enteric infection in newborn mice. No such infection has ever been recorded in any of the normal mouse colonies maintained in these laboratories. Aetiological studies of the murine hepatitis viruses have been hampered by the very fact of their latency (18) but now it would appear that, by chance, we have produced a most useful tool, the neonatally thymectomized mouse, that could profitably be used in future investigations. Why a thymectomized host should provide such a favourable milieu for this particular virus is not yet known but, as far as this publication is concerned, interest centres on the part played by the virus in the premature death of mice thymectomized at birth.

While there is little doubt that the intact newborn recipients of necrotic tissue from thymectomized wasting donors die with gross liver damage it is difficult to ascribe the same cause of death to the donors themselves since only a few show severe damage and at least half of all the thymectomized mice waste and die without liver lesions. This might imply that the virus is only secondarily hepatotrophic and that its primary attack is made elsewhere but the histological evidence would seem to refute this interpretation. Since the virus is normally latent in mice of the strains used (18), it is logical to suggest that a symbiotic host-virus relationship is disturbed by neonatal thymectomy and that the virus slowly increases in concentration until it reveals its presence by causing liver necroses in some of the mice, particularly those which waste and die relatively late. A slow build-up of infection could explain why virus cannot be detected in healthy thymectomized mice and those animals which waste and die without liver lesions and whose tissues cannot be successfully passaged could be presumed to have died during the period in which the titre of virus is still very low. It is tacitly assumed in this hypothesis that the death of thymectomized animals is not due to liver damage but another explanation is suggested by the fact that, while the incidence of liver lesions may vary, wasting occurs inevitably in all neonatally thymectomized mice. In this case a low-grade hepatitis could be held responsible for the physical deterioration and so indirectly be the cause of death. This alternative is difficult to prove or disprove because passage of the disease from healthy thymectomized mice and from wasting mice without lesions has been unsuccessful and we have not yet been able to simulate the curious gait, hunching, and physical deterioration in intact animals given small doses of the virus.

Thus, while there seems little doubt that neonatal thymectomy in some way alters a stable host-virus relationship we hesitate to define the part played by the hepatotrophic virus in the wasting syndrome and prefer, in the present stage of our investigations, to regard it as a complicating but incidental infection which is not the primary cause of death.

SUMMARY

Inbred (C57BL; C3H/Bi), hybrid (C57BL × C3H/Bi), and outbred (TO) mice thymectomized within 24 hours of birth develop wasting symptoms and die prematurely and a proportion of these animals have pathological changes in the liver. The incidence of the liver lesions varies according to the strain of mice used and the lesions tend to occur in animals dying comparatively late.

These lesions were shown, by passage of tissue suspensions and of cell-free liver extracts, to be due to a hepatotrophic virus probably mouse hepatitis virus-1 (MHV-1). The part played by the hepatotrophic virus in the premature death of thymectomized mice is discussed but, although neonatal thymectomy

apparently alters a normally stable host-virus relationship, it is not thought that the virus is primarily responsible for the death of its host. The role of this virus in the production of the physical wasting is also considered to be problematic.

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BIBLIOGRAPHY

1. Parrott, D. M. V., Strain variation in mortality and runt disease in mice thymectomized at birth, *Transplant. Bull.*, 1962, **29,** 102.
2. Parrott, D. M. V., and East, J., Role of the thymus in neonatal life, *Nature*, 1962, **195,** 347.
3. Miller, J. F. A. P., Immunological function of the thymus, *Lancet*, 1961, **2,** 748.
4. Martinez, C., Kersey, J., Papermaster, B. S., and Good R. A., Skin homograft survival in thymectomized mice, *Proc. Soc. Exp. Biol. and Med.*, 1962, **109,** 193.
5. Parrott D. M. V., and East, J., Studies on a fatal wasting syndrome of mice thymectomized at birth, *in* The Thymus in Immunobiology, (R. A. Good, editor), New York, Harper and Row, 1963, in press.
6. Miller, J. F. A. P., Effect of neonatal thymectomy on the immunological responsiveness of the mouse, *Proc. Roy. Soc. London, Series B*, 1962, **156,** 415.
7. East, J., and Parrott, D. M. V., Operative techniques for newborn mice using anaesthesia by cooling, *J. Endocrinol*, 1962, **24,** 249.
8. Gledhill, A. W., Dick, G. W. A., and Andrewes, C. H., Production of hepatitis in mice by the combined action of two filterable agents, *Lancet*, 1952, **2,** 509.
9. Niven, J. S. F., Gledhill, A. W., Dick, G. W. A., and Andrewes, C. H., Further light on mouse hepatitis, *Lancet*, 1952, **2,** 1061.
10. Gledhill, A. W., Dick, G. W. A., and Niven, J. S. F., Mouse hepatitis virus and its pathogenic action, *J. Path. and Bact.*, 1955, **69,** 299.
11. Cheever, F. S., Daniels, J. B., Pappenheimer, A. M., and Bailey, O. T., A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. I. Isolation and biological properties of the virus, *J. Exp. Med.*, 1949, **90,** 181.
12. Morris, J. A., A new member of the hepatoencephalitis group of murine viruses, *Proc. Soc. Exp. Biol. and Med.*, 1959, **100,** 875.
13. Nelson, J. B., Acute hepatitis associated with mouse leukaemia. I. Pathological features and transmission of the disease, *J. Exp. Med.*, 1952, **96,** 293.
14. Braunsteiner, H., and Friend, C., Viral hepatitis associated with transplantable mouse leukaemia. I. Acute hepatic manifestations following treatment with urethane or methylformamide, *J. Exp. Med.*, 1954, **100,** 665.
15. Kassel, R., and Rottino, A., Problems in the production of leukaemia with cell-free extracts, *Cancer Research*, 1959, **19,** 155.

16. Manaker, R. A., Piczak, C. V., Miller, A. A., and Stanton, M. F., A hepatitis virus complicating studies with mouse leukaemia, *J. Nat. Cancer Inst.*, 1961, **27,** 29.
17. Rowe, W. P., Hartley, J. W., and Capps, W. I., Mouse hepatitis as a highly contagious, prevalent, enteric infection of mice, *Proc. Soc. Exp. Biol. and Med.*, 1963, **112,** 161.
18. Gledhill, A. W., and Niven, J. S. F., Latent virus as exemplified by mouse hepatitis virus (MHV), *Vet. Rev. and Annotations.*, 1955, **1,** 82.

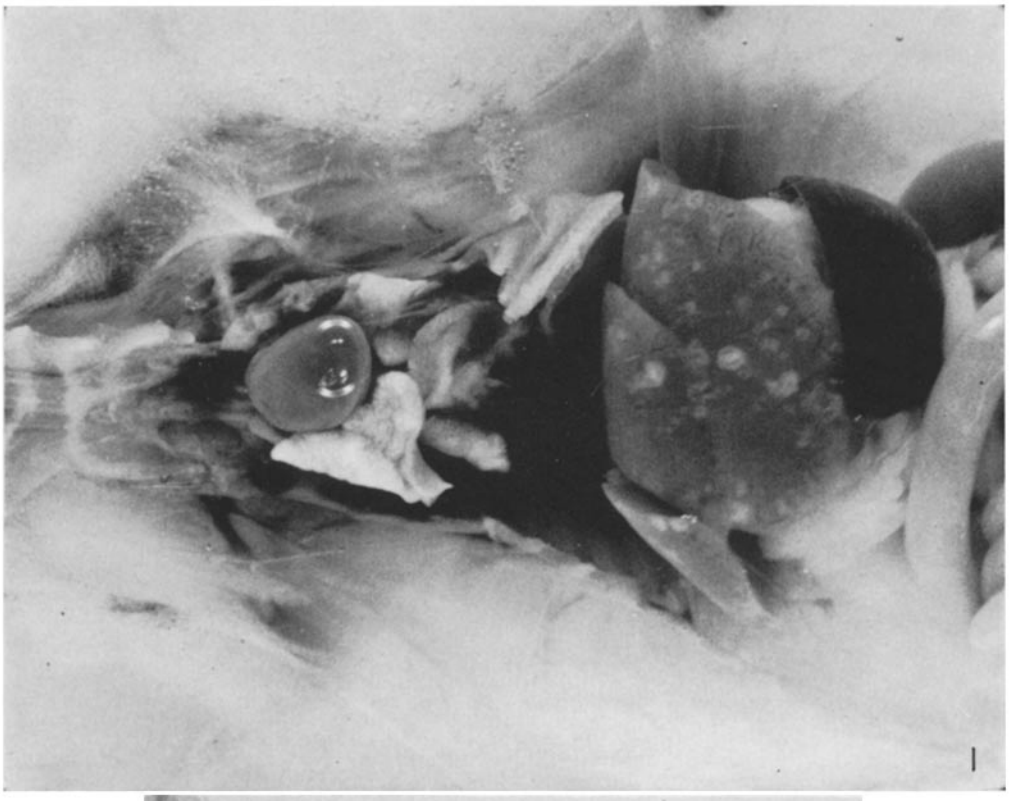
EXPLANATION OF PLATES

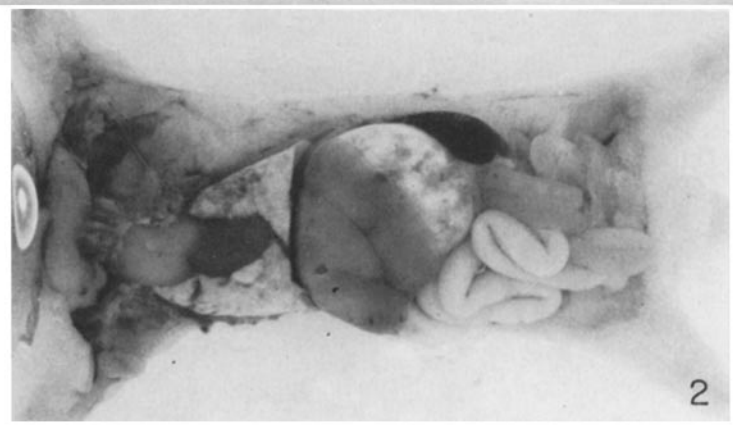
PLATE 90

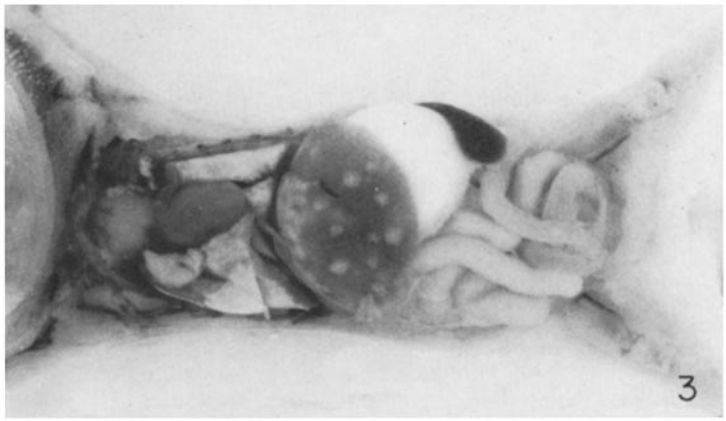
FIG. 1. Autopsy appearance of female TO donor mouse thymectomized at birth and wasting when killed at 56 days. Note white necrotic foci on liver. × 3.

FIG. 2. Autopsy appearance of intact untreated female TO mouse aged 6 days. Litter mate of animal shown in Fig. 3. × 3.

FIG. 3. Autopsy appearance of intact female TO recipient mouse aged 6 days and killed 5 days after injection of necrotic liver suspension from a thymectomized wasting adult donor. Note that white focal lesions on the liver are becoming confluent. × 3.







(East *et al.*: Hepatotrophic virus in thymectomized mice)

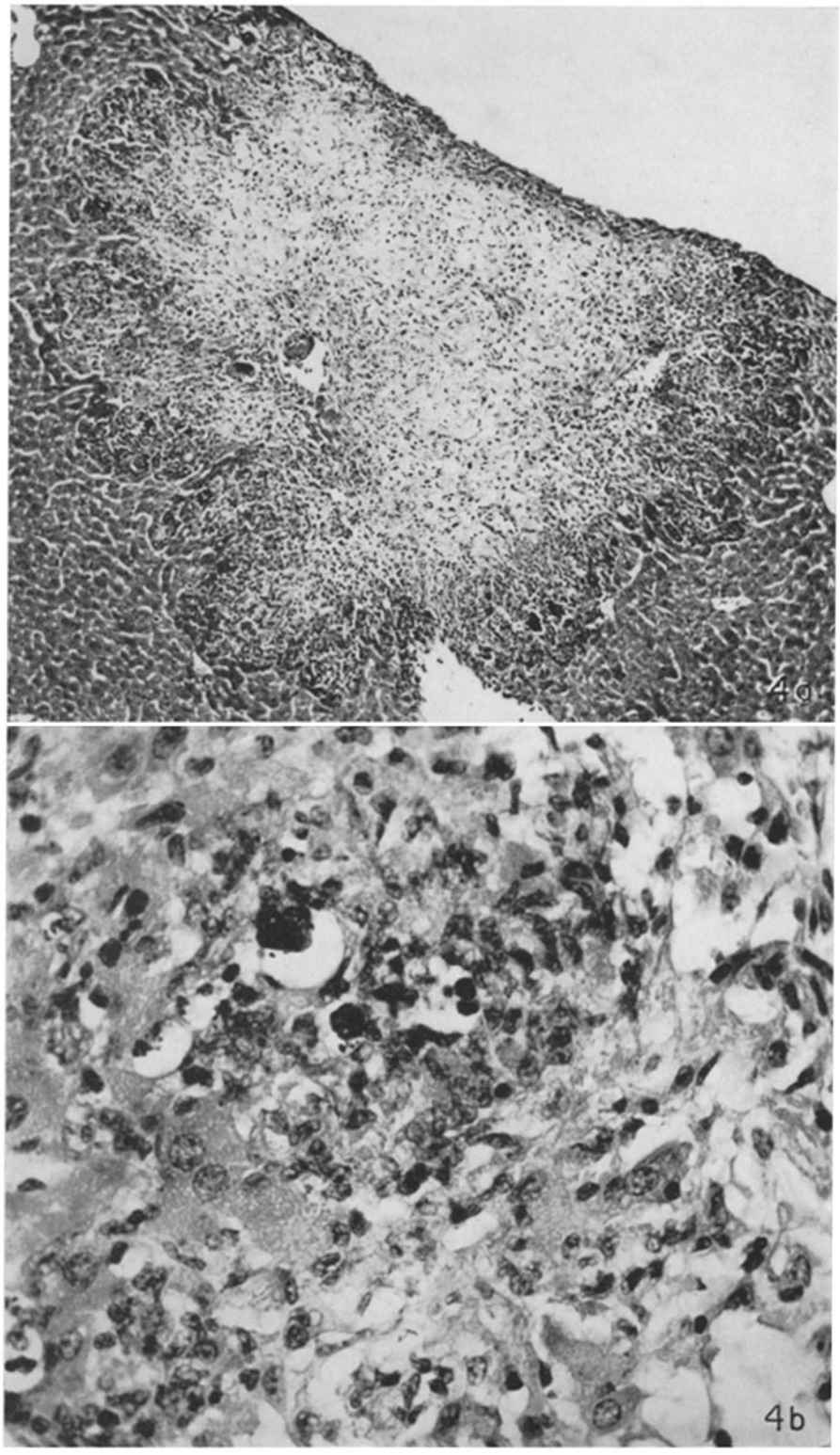
PLATE 91

All the microscopic specimens were stained with haematoxylin and eosin.

FIGS. 4 *a* and 4 *b*. Liver of female C3H donor mouse thymectomized at birth and wasting when killed at 50 days.

FIG. 4 *a*. Area of necrosis containing giant cells adjacent to normal liver tissue. × 100.

FIG. 4 *b*. Higher magnification of Fig. 4 *a* showing necrotic liver cells with aggregates of hyperchromatic nuclear debris. × 500.



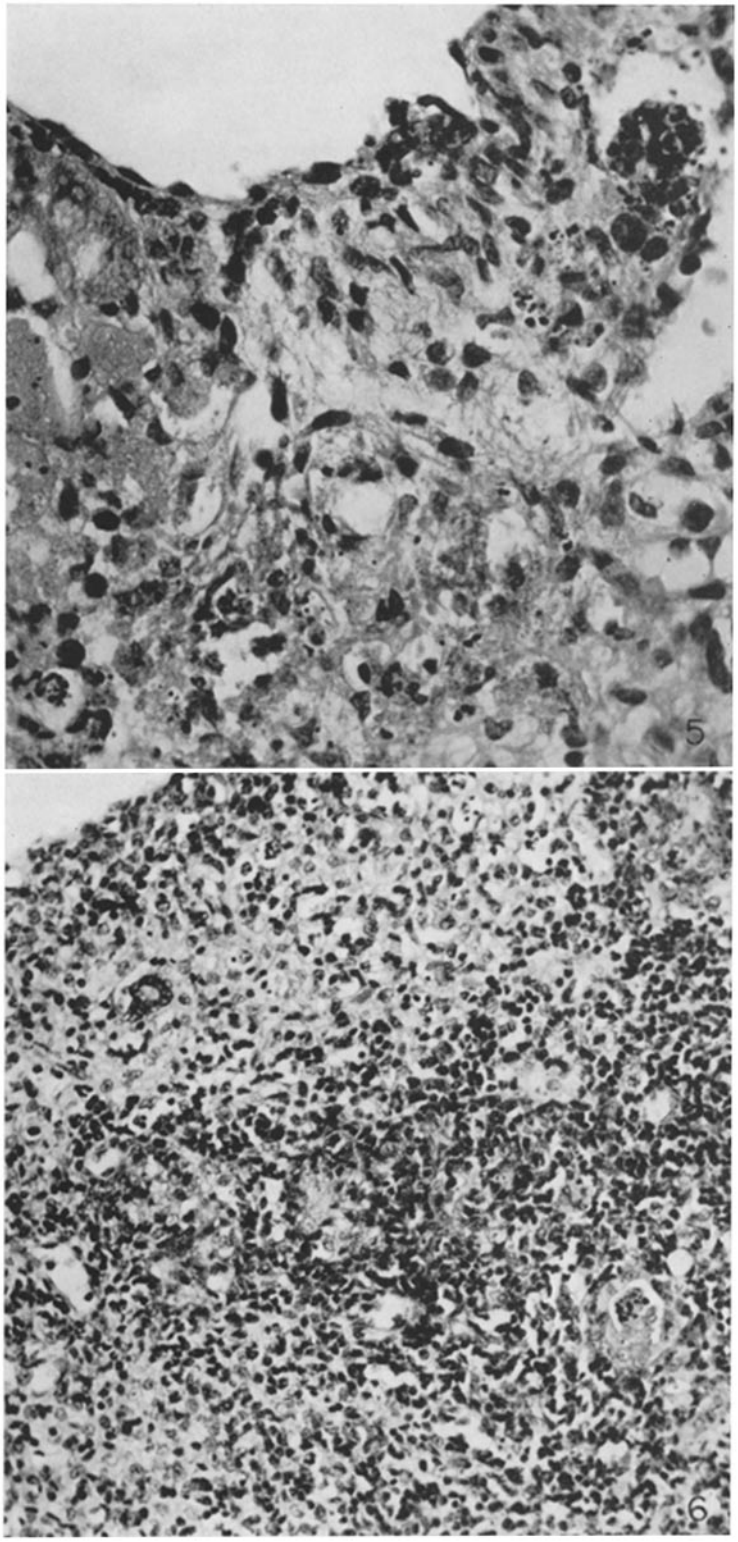
(East *et al.*: Hepatotrophic virus in thymectomized mice)

PLATE 92

Histological appearance of organs from intact recipient baby mice inoculated with suspensions of necrotic liver from neonatally thymectomized wasting adult donors.

FIG. 5. Liver of intact male C3H recipient mouse aged 7 days and killed 6 days after inoculation. Note extensive destruction of parenchyma with oedema, slight cellular infiltration and inter- and intrasinusoidal debris. × 500.

FIG. 6. Thymus of intact female C3H recipient mouse aged 11 days and killed 8 days after inoculation. Note diminution of lymphocytes, focal collections of debris, and one giant cell. × 250.

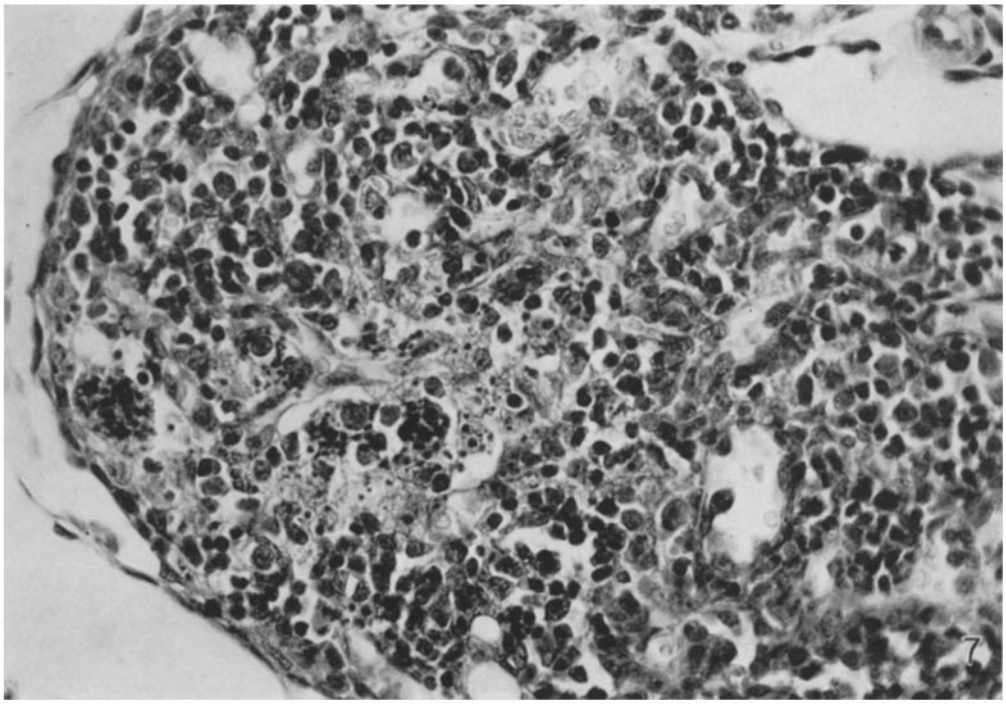


(East *et al.*: Hepatotrophic virus in thymectomized mice)

PLATE 93

Histological appearance of organs from intact recipient baby mice inoculated with suspensions of necrotic liver from neonatally thymectomized wasting adult donors.

FIG. 7. Inguinal lymph node of intact female TO recipient mouse aged 5 days and killed 4 days after inoculation. Note foci of nuclear debris. × 500.



(East *et al.*: Hepatotrophic virus in thymectomized mice)