Neonatal Intestinal Lipidosis in Mice

An Inherited Disorder of the Intestinal Lymphatic Vessels

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In 1964 Herbertson and Wallace described an inherited form of chylous ascites developing in some 15% of newborn mice heterozygous for the gene *ragged*, *Ra*. The affected animals appeared normal at birth but, after suckling, milk-like fluid containing abundant small fat droplets began to collect in the peritoneal cavity. The wall of the small intestine was considerably paler and thicker than usual, the submucosa becoming a broad layer distended with innumerable globules of lipid. In addition, the mesenteric lymphatic vessels draining the small intestine were grossly distended with chyle. The disorder was thought to be caused by defective lymphatic drainage from the small intestine, but though probably due to an obstruction to lymph flow near the root of the mesentery, a thoroughly convincing morphological explanation was not established.

Present Study

In 1962, during the maintenance of strain A mice at the Department of Genetics at Cambridge, a spontaneous mutation occurred, giving a somewhat similar disorder of newborn mice, but in the absence of the gene *Ra*. Investigation has shown that the heterozygote has imperfect penetrance and the homozygote is antenatally lethal. The gene is in a different linkage group from *Ra*.

The new disorder is also apparently caused by defective transport of fat from the small intestine. The affected mice appear normal at birth, but some hours after they have begun to suckle, parts of the small intestine become china-white in colour. This change can readily be seen through the abdominal wall of the living animal during the first 4 days of life. The defect is usually visible within 1 or 2 days of age, but occasionally an apparently normal mouse shows the defect at 2 to 4 days. In most defective animals there is no excess of fluid in the peritoneal cavity, but in a few a trace of chylous fluid is found

between the loops of the intestine and other viscera when an animal is dissected after death. The abnormal china-white portions of the intestinal wall are much thicker than normal, and contain abundant fat, mostly in droplet form. At first the fat droplets lie freely in the intestinal wall but later most are engulfed by macrophages.

Most of the affected animals thrive and gain weight at about the usual rate, and after 3 or 4 weeks their intestines become structurally normal. A small number, however, though of normal weight at birth, fail to gain weight in the usual fashion during the first 10 days. Of these, a very few die before 5 days; most improve rapidly, but even when adult they tend to be somewhat small and underweight, despite the apparent recovery of their intestines. The greatest mortality is between 2 days and 4 weeks, but this is only of the order of 10% of genetically mutant animals; death appears to be due to their inability to compete for milk with their more active unaffected normal litter-mates. Affected animals surviving weaning are on the whole less fertile and have smaller litters than their litter-mates, but the mutant presents no great difficulties in routine maintenance.

An exact counterpart of this disorder does not seem to have been discovered in man or other mammals, and in the absence of an established name we have used the rather cumbersome but descriptive term 'neonatal intestinal lipidosis'; the mutant responsible has been given the genetic symbol *Nil*, an abbreviation of this term (Wallace, 1965).

This account describes preliminary genetic investigations (M. E. Wallace) and morbid anatomical and histological features (B. M. Herbertson).

Genetic Investigations

Classification. Classification as 'affected' or 'unaffected', of the progeny of all matings, was routinely carried out daily or almost daily from birth until 4-5 days old. Dissection and macroscopical examination at 4

days revealed that only a very few mice classified alive as unaffected were in fact affected, while dissection at 4-14 days altered the classification even more rarely. At first, therefore, dissection was used whenever classification was doubtful, but as experience increased, doubt diminished and dissection became unnecessary. However, in those investigations where accuracy must be maximal (e.g. concerning the viability of homozygotes, and linkage relations), classification was done both in the live and in the dissected animal.

Dominance and Penetrance. The first affected animal appeared in the 112th generation of sibmating of strain A/FaCam.* It was crossed to a normal mouse from strain AG/Cam,* and affected progeny from this and from each of four further generations were crossed to this strain. Affected mice from the fifth and subsequent crosses to AG were thus virtually isogenic with this strain; mice from this source were designated D/AG and used in the present investigation and some of those described below.

The appearance of affected mice in the first generation indicates dominance, and their appearance in each of the subsequent generations indicates a single gene responsible for the condition. All sibs and other relatives of the first affected mouse, and their descendants within strain A, for several generations, were examined, but no further affected ones were seen. It is concluded that the first mouse was the result of a spontaneous mutation.

The matings in the crosses to AG can be denoted $Nil/+ \times +/+$ (where + is the normal allele of *Nil*). Pooling the data, they give 80 affected and 167 unaffected progeny. This is significantly different from the 1:1 expected from a simple dominant ($\chi^2=30.6$ for 1 d.f., probability is less than 0.001). The likeliest explanations are: (a) inviability of *Nil/+* before birth or before classification, and (b) imperfect penetrance of *Nil/+* (i.e. the phenotypically normal appearance of some individuals whose genotype is *Nil/+*).

To discriminate between the two explanations, 11 unaffected mice from $Nil/+ \times +/+$ matings were crossed to $+/+$ from AG (or other sources). Of these, 5 produced some affected progeny, thus showing them-

selves to be genotypically *Nil/+*, and the other 6 produced only unaffected ones, thus confirming their genotype as $+/+$. Thus, roughly half the phenotypic unaffected were *Nil/+*. Since only about one-quarter of the 167 observed unaffected progeny (i.e. 42) require to be genotypically *Nil/+* in order to restore the expected 1:1, imperfect penetrance adequately accounts for the observed incidence of affected progeny. Thus inviability of *Nil/+* before classification is trivial, a conclusion more extensively proven below. The degree of impenetrance of *Nil/+* is $42/(80+42)=34\%$.

As a check on the certainty of classification of the supposedly *Nil/+* progeny 15 affected animals from $Nil/+ \times +/+$ matings were also crossed to $+/+$ from AG (or other sources). All 15 produced some affected progeny. Thus the experienced person can be certain that no $+/+$ are accidentally classified as affected.

From the above considerations, and from the antenatal lethality of *Nil/Nil* homozygotes (see below), it is clear that all affected animals are *Nil/+*, but unaffected ones are genotypically $+/+$ or *Nil/+*. To ensure the genotype of backcross matings ($Nil/+ \times +/+$) here and elsewhere, an affected animal was always mated to a normal one from a stock not containing *Nil*. Intercrosses ($Nil/+ \times Nil/+$) similarly consisted of two affected animals from a backcross, until lethality of homozygotes was proved.

Lethality of Homozygotes. During the process of isogenesis with strain AG, several intercrosses $Nil/+ \times Nil/+$ were made. It was noticed that albinism, *c* (linkage group I), homozygous in strain A where *Nil* originated, continued to segregate in these intercrosses, but with a very low frequency. Continued segregation suggested that albinism was closely linked with *Nil*, and the low frequency suggested that *Nil/Nil* was lethal. Linkage was investigated and the recombination value was found to be about 6% (Wallace, 1967).

To test the lethality of *Nil/Nil*, affected albinos ($Nilc/+c$) were crossed to normal coloured mice ($+C/+C$) from strain AG and elsewhere; their affected coloured progeny ($Nilc/+C$) were crossed *inter se*, forming double intercrosses, and to normal coloured animals of genotype $+c/+C$, forming single intercrosses. The results are given in Table I. The double intercrosses

TABLE I
INTERCROSSES INVOLVING *Nil* AND ALBINISM

Parents	Phenotypes of Progeny							Coloured: Albino	χ^2 testing 3:1
	Normal Coloured	Normal Albino	Affected Coloured	Affected Albino	Uncertain* Coloured	Uncertain* Albino	Total		
A $Nilc/+C \times Nilc/+C$	393	6	216	17	15	3	650	624:26	152.88 $p < 0.001$
B $Nilc/+C \times +c/+C$	80	14	11	15	0	0	120	91:29	0.043 $p > 0.7$

* These mice died before 4 days old. Their *Nil* phenotype is therefore uncertain. Their albino phenotype is known by eye-colour.

(Table I:A) are expected to give one-quarter albinos of which most are *Nilc/Nilc* and die if *Nil/Nil* is lethal. The single intercrosses (Table I:B) are expected to give one-quarter albinos of which most are *Nilc/+c* and live.

The very significant deficiency of albinos in the double intercrosses as compared with the single intercrosses confirms the general lethality of *Nil/Nil*.

However, since *Nil/+* sometimes have a normal phenotype, it is conceivable that a very few *Nil/Nil* do also and that they then survive.

This can be tested as follows: estimate how many albino zygotes were formed from the double intercrosses, and how many of them are expected to be other than *Nil/Nil* and so to live; and then compare this expectation with the observed number that lived. If there is agreement, there are no grounds for supposing that any *Nil/Nil* survive.

Assuming from Table I that 624 coloured zygotes were formed, and that the zygotic ratio 3 coloured: 1 albino was realized, 208 albino zygotes must also have been formed. Of these, the expected number of *Nil/Nil* is the product of the frequencies with which each parent's albino gametes have not recombined with *Nil*, namely $(100\%-6\%)^2$, multiplied by 208. This is 183.79. The expected number of albinos of genotype other than *Nil/Nil* is then $208-183.79=24.21$. The number of living albinos observed is 26. The agreement is thus very close, and there are no grounds for supposing that any of the albinos living after birth were *Nil/Nil*.

As further confirmation of *Nil/Nil* lethality, 11 albinos (mainly affected) from the double intercrosses were mated to *+/+* from AG and other sources; their unaffected progeny were mated again to *+/+* and allowed to rear at least 12 progeny. Of the 11, 2 males were infertile. 5 males and 3 females showed themselves to be *Nil/+* by producing an incidence of affected progeny similar to, and not exceeding, that obtained previously from *Nil/+*, and, in the case of the males, by their unaffected offspring's production of unaffected only. The remaining female proved to be *+/+*, by her production of 46 progeny, all unaffected, and by her 2 daughters' production of unaffected only. Thus no mouse was eligible as *Nil/Nil*. Moreover, the finding of 8 *Nilc/+c* and one *+c/+c* agrees closely with the numbers expected on the basis of 6% recombination and of lethality of *Nil/Nil*; these numbers, obtained in a similar manner to that above, are 8.72 and 0.28, respectively. χ^2 testing the fit of observation to expectation is 2.44 for 1 d.f., probability 0.3.

Thus there are no grounds for supposing that any *Nil/Nil*, whether phenotypically affected or not, survive to maturity.

It is conceivable of course that selection for impenetrance of *Nil/+* could result in so mild an expression of the homozygote that it could survive. A precedent for this lies in the selection for long tails in the heterozygotes of Danforth's short-tail, *Sd/+*; this resulted in the survival for over a week of 2 homozygotes, otherwise neonatally lethal (Fisher and Holt, 1944). One further *Sd/Sd* lived to maturity and proved her genotype, on

being crossed to unselected normals, by producing all short-tail progeny (M. E. Wallace and M. MacNeil, unpublished). No such selection experiment has been attempted on *Nil/+*. It is noteworthy, however, that in one stock of ragged, where the heterozygotes had no incidence of chyloous ascites, and homozygotes were neonatally lethal, a stock of viable adult *Ra/Ra* was reared after selection for viability alone, and that this stock was then found to have a lowered expression of the generalized oedema typical of unselected homozygotes (Slee, 1957).

It remains to discover at what stage of development *Nil/Nil* homozygotes die. Preliminary investigations by P.A. Screen (City of Coventry Training College, personal communication), on the death-rate of albino embryos from *Nilc/+C* $\times$ *Nilc/+C* matings, indicate that they die at about the stage when albinism is itself classifiable by eye-colour, namely 11 days' gestation.

Penetrance and Milk Supply. The impenetrance of *Nil/+* in the single intercrosses (Table I:B) is 56.6%, i.e. higher than in the material obtained during isogenesis with AG (34%). This is probably due to the use of *+c/+C* mates from a source other than AG. Impenetrance has in fact been observed to vary according to different stocks, being as low as 30% in one stock and as high as 80% in another (Wallace, 1967). Whether this variation is controlled by the genotype of the mother or the genotype of the offspring is not certain; but a crude test of the importance of milk supply was thought likely to throw some light on the mechanism of control.

It had been noticed that strain JU/FaCam females give a high degree of impenetrance and that their litters are large enough to allow removal of half the young without detriment to the survival of the remainder. *Nilc/+C* males from D/AG were mated to some 8 females of this strain. Since JU mice are albino (*+c/+c*), their albino young, by these males, are expected to be mainly (94%) *Nil* heterozygotes, and their coloured young mainly (94%) normal, i.e. *+/+*. Removal of coloured young in a litter is thus expected to increase the milk supply to the remaining *Nil/+*. Comparison of the incidence of *Nil* in albino young in intact litters, with that in albino young in litters where coloured sibs have been removed, is thus a crude test of the effect of milk supply on impenetrance.

The 8 JU females were allowed to rear intact one or two litters each. In their next one or two litters, coloured young were removed at under 4 days old; in most of these litters removal was at 9–10 a.m., and in the others it was between 10 a.m. and 12 noon. The results are given in Table II.

The significant decrease in impenetrance in treated litters over that in the intact ones indicates that a sudden increase in milk supply does increase the proportion of *Nil/+* genotypes which show the affected phenotype. The significant decrease in impenetrance when removal is after 10 a.m. as against removal before 10 a.m. probably has the same explanation. A separate small study at the same time of the year showed that weight increase per hour in intact pure strain JU litters is twice as big in

TABLE II
EFFECT ON PENETRANCE OF REDUCING COMPETITION FOR MILK

Genotype of Parents <i>Nil c/c</i> × <i>C</i> × <i>+c/c</i> × <i>c</i>	Phenotypes of Albino Progeny			Contingency χ^2 for 1 d.f. with Yates' Correction	Probability
	Affected	Normal	Impenetrance %		
Intact litters	29	93	76	6.08	< 0.02
Reduced litters	50	77	61		
Litters reduced before 10 a.m.	31	67	70	9.38	< 0.01
Litters reduced after 10 a.m.	19	10	34		

the two-hour period 10–12 noon as it is in the remaining 22 hours; it may be conjectured that this is because the females settle down to suckle during this period after their nocturnal activity, but clearly a more precise study is needed to be sure of this.

Viability of *Nil/+* Heterozygotes. After the finding of linkage of *Nil* with the albino locus, tests were carried out with other markers in linkage group I. The results of three-point backcrosses showed that *Nil* lies between albinism and pink-eyed dilution, *p*; its recombination value with *p* is about 3% (Wallace, 1967). Impenetrance of *Nil* in this stock was 40%.

In these backcrosses, the part of the heterozygotes' genotype involving *Nil* and *p* was as follows: *NilP/+p*. Mated to *+p/+p*, they give progeny of which the dark-eyed phenotypes are expected to be mainly (97%) *Nil/+* and the pink-eyed mainly (97%) *+/+*. Since the *p/p* genotype is neonatally fully viable in the absence of *Nil* (M. E. Wallace, unpublished), inequality in the observed number of dark-eyed and pink-eyed progeny represents unequal viability of *Nil/+* and *+/+*. An exact measure of the death-rate of the *Nil/+* genotype (including those phenotypically unaffected) at different ages may therefore be obtained by observing the age at death of dark-eyed and pink-eyed progeny; these observations are given in Table III.

TABLE III
SEGREGATION AT THE *p* LOCUS IN BACKCROSSES
Nil P/+p × *+p/+p*

Age at Death (days)	Observed Numbers			Estimated Loss of <i>P</i> %	
	<i>P</i>	<i>p</i>	Total	Absolute	Relative
After 14	609	713	1322	81.2	6.9 1.7 } 8.6 0
Between 4 and 14	73	21	94	9.7	
Between 0 and 4	29	16	45	3.9	
Before birth	39*	0*	39	5.2	
Estimated total of zygotes	750	750	1500	100.0	

* Estimated by subtraction from the total of zygotes.

The first line of observations comprises mice dying or killed after 14 days, at which age classification of all loci was complete and litters routinely destroyed. The second line comprises mice classified for *Nil* and *p* by 4 days and dying before 14 days. The third line com-

prises mice classified for *p* soon after birth and dying before *Nil* was classified at 4 days. The 'estimated total of zygotes' in the last line is obtained by summing the *p* mice and assuming that none died before birth, giving a total of 750 *p* mice, and by assuming that an equal number of *P* zygotes were formed, giving 750 *P* mice. The number 39 in the fourth line is then 750 less the rest of the figures in the same column. The absolute loss of *P* for each age is then the observed number for that age, divided by 750, expressed as a percentage.

The figure 5.2% represents an upper limit for the prenatal period. There is in fact justification for thinking that this could be 0% (as would be expected if *Nil/+* suffer in no way from their disorder until after suckling), for the totals for post-natal deaths are 750–39 *P* and 750 *p*; these figures do not differ significantly from 1:1 ($\chi^2 = 1.01$ for 1 d.f., probability less than 0.3), and so may be taken as zygotic figures. The other percentages may thus be very slightly underestimated.

Estimates for absolute loss include chance deaths as well as deaths specifically due to the *Nil/+* genotype. If the loss of *p* mice is taken as an indication of chance deaths, an estimate of the loss of *Nil/+* relative to chance deaths may be obtained by subtracting the observed number of *p* from the observed number of *P* and dividing by 750. The resulting percentages are given in the last column of the Table. It is thus clear that relative loss of *Nil/+* before 14 days is not less than 8.6%; if the maximum loss before birth of 5.2% is added to this, we have the maximum loss at 13.8%.

A separate survey of deaths of affected *Nil/+* mice between 2 days old and 4 weeks (unpublished) shows this to be about 10%.

The possibility that death-rate depends on penetrance has not been studied. Where impenetrance is very low, the death-rate of *Nil/+* (affected plus unaffected) would be expected to be higher, but a general impression has been gained from different stocks that expression is not so variable between stocks that a death-rate outside the range 5–20% of affected *Nil/+* would be observed.

An exact measure of death-rate after 14 days has not been made. Though the peak loss is certainly at 4–14 days, there is an appreciable loss of fertile mated animals; adult *Nil/+* tend to die younger than *+/+*.

Interaction with Ragged. Where mutants have very similar developmental effects, it is commonly found

that the presence of one enhances the effect of the other, and that modifiers of one modify the other; thus, among the spotting genes in mice, a double mutant has more extensive spotting than the sum of the effects of the mutants singly, and a genetic *milieu* such as a particular inbred line which enhances the effect of one, enhances the effect of the other also. Since both *Nil* and *Ra* concern the lymphatic system, a study of their interaction would be expected to throw some light on their developmental relation. (It must be pointed out here that chylous ascites in ragged is not due to a separate gene closely linked to ragged, as was at first supposed, but is a pleiotropic effect of the *Ra* gene itself: Wallace, 1966.)

Two stocks of *Ra*/+ were used. In one, the incidence of chylous ascites in *Ra*/+ was 0%, in the other 17%. Ragged mice from each were crossed to *Nil*/+ from D/AG, where the impenetrance of *Nil* was 34%. In each case, ragged progeny, thought from external scrutiny to show chylous ascites and intestinal lipidosis, were crossed to normal mice of the stock from which the *Ra* gene had come. From their progeny, in each case, a second backcross of supposed *Ra*/+, *Nil*/+ was made to the stock of the *Ra* gene's origin. Segregation from each cross confirmed the presence of *Nil*, which would otherwise have been uncertain. Indeed, even with dissection, the presence of *Nil* in an animal showing chylous ascites might have been uncertain, and for this reason classification of all progeny was confined to noting whether or not their abdomens showed a 'milky' appearance.

The frequencies of the four phenotypes as between the three crosses to the '0%' stock were homogeneous, and so are pooled; so also for the three crosses to the '17%' stock. These data are given, as segregation '0%' and segregation '17%' respectively, in Table IV.

TABLE IV
INTERACTION OF *Nil* AND *Ra* IN CROSSES
Ra/+, *Nil*/+ × +/+, +/+

Segregation	Phenotypes of Progeny				Total
	Normal	'Milky'	Ragged	Ragged-'milky'	
0%*	166	83	154	101	504
17%†	137	32	83	82	334
Contingency χ^2 ‡	9.76		3.75		
Probability	<0.01		≈0.05		
Segregation	Impenetrance of <i>Nil</i>		Incidence of 'milky'		
0%*	33%		40%		
17%†	61%		50%		

* The normal parent came from the stock in which the incidence of chylous ascites was 0%.

† The normal parent came from the stock in which the incidence of chylous ascites was 17%.

‡ The contingency χ^2 has 1 d.f. and Yates' correction.

Homogeneity between generations confirms that *Nil* and *Ra* are unlinked, as was expected from their location in different linkage groups (I and V respectively); the zygotic segregation in these data is thus expected to be:

equal numbers of +/+, +/+; *Ra*/+, +/+; +/+, *Nil*/+; and *Ra*/+, *Nil*/+.

The impenetrance of *Nil* is therefore accurately estimated from the segregation of 'milky' within the non-*Ra* mice. The incidence of 'milky', estimated from the segregation of 'milky' within the *Ra*/+ mice, is a measure of the undistinguished effects of *Ra* and *Nil*.

It is striking that the impenetrance of *Nil* is the same in the '0%' stock as it was in the stock of *Nil*'s origin (33% and 34%, respectively), but that it is significantly greater in the '17%' segregation (62%). It is clear therefore that modifiers which enhance the incidence of chylous ascites in *Ra*/+ do not enhance the penetrance of the *Nil* gene. It must be supposed either that they diminish it, or that there are separate modifiers in the '17%' stock which diminish it.

If it is assumed that the impenetrance of *Nil* is the same among the raggeds as among the non-raggeds, and that the incidence of chylous ascites in the '0%' segregation is in fact 0%, then the expected ratio of non-'milky' to 'milky' among the raggeds is 170:85. With the observed figures at 154:101, it is clear that this is not the case (the probability of agreement is less than 5%). It must be concluded therefore either that *Ra* enhances penetrance in *Nil*/+ or that *Nil* enhances the incidence of chylous ascites in *Ra*/+.

However, the incidence of 'milky' in the '17%' segregation where the impenetrance of *Nil* is high, is substantially greater than in the '0%' segregation. If *Ra* enhances the penetrance of *Nil*, it has its greatest effect where modifiers depress *Nil* penetrance the most. Since one would expect a mutant and its modifiers to have the same effect on another mutant, this seems unlikely. A more likely explanation therefore seems to be that *Nil* enhances the incidence of chylous ascites in *Ra*/+. It is interesting to note in this context that the gene fidget, *fi*, whose skeletal effects have been thoroughly examined (Truslove, 1956) has no known effect on the lymphatic system, yet in fidget mice the incidence of chylous ascites in *Ra*/+ is twice as great as it is in non-fidget mice of the same stock (Wallace, 1966). It seems that the incidence of chylous ascites in *Ra*/+ reflects a failure in canalization whose cause is not very specific.

Morbid Anatomy and Histology

Materials and Methods. Forty-one young mice with neonatal intestinal lipidosis ranging from about 20 hours to 26 days, and 54 normal mice of both sexes and similar ages, were killed with a mixture of chloroform and coal gas and dissected shortly after death. A particular study was made of the distribution of the intestinal lesions and of the condition of the related blood and lymphatic vessels. After detailed examination with a dissecting microscope the gastro-intestinal tract was removed and, with blocks of other tissues and the remainder of the carcass, was fixed in 4 per cent formaldehyde saline. The technique of fixing the gastro-intestinal tract varied but on many occasions it was gently pinned on to a cork sheet and fixed *in toto*. To ensure satisfactory preservation of the gastro-intestinal

mucosa, formaldehyde saline was often introduced into the lumen of the stomach and intestines, care being taken to avoid undue distension. In some instances lengths of normal and affected intestine were opened longitudinally and the villi on the mucosal surface examined with a dissecting microscope both before and after fixation. Paraffin and frozen sections of appropriate tissues were then prepared, the methods being similar to those used in a previous investigation (Herbertson and Wallace, 1964).

Eleven adult mice, 5 to 18 months old, which had suffered from neonatal intestinal lipidosis and had later been used for breeding purposes, were similarly killed and examined.

Results

In most of the abnormal mice the only observable defect was the altered colour of the small intestine. A few of the more severely affected lost weight during the first 4 or 5 days but they appeared healthy throughout life in all other respects. An effect of the impaired weight gain was a delay in the deposition of subcutaneous adipose tissue; this allowed a better and longer-term view of the intestinal lesions than in the less affected animals.

Small Intestine

Macroscopical Changes. The principal macroscopical change was a whitening of parts of the small intestine. In normal newborn mice the wall of the stomach and intestines is thin and the colour of

different parts of the gastro-intestinal tract is largely determined by their contents. The stomach and proximal part of the duodenum of a normal suckling mouse contains milk and tends to be yellowish white whereas the rest of the small intestine with its bile-stained contents is usually a deeper orange-brown colour (Fig. 1). In the affected mice portions of the small intestine become a china-white colour usually within 48 hours of birth. This change was impressive and in the vast majority of instances could readily be seen through the abdominal wall of the living animal (Fig. 2). The parts of the small intestine affected varied from animal to animal. Sometimes almost the whole of the small intestine from the pylorus to the caecum was involved, but more frequently only certain parts were affected (Fig. 3). Commonly the affected part was abnormal around its whole circumference, but sometimes only a segment was involved and in such instances the abnormal area was usually on the side away from the attachment of the mesentery. Occasionally one or more brilliant red spots (about 1 mm. diam.) were present on the anti-mesenteric surface of an affected part of the intestine (Fig. 2). If more than one were present the individual spots were usually at least 0.5 cm. apart. Though these spots were most readily seen when the abdominal cavity had been opened during post-mortem examination, they could sometimes be seen through the intact abdominal wall during life. The youngest

FIG. 1. Normal mouse (5 days old; body weight = 3.2 g.) viewed from the ventral surface. (a) Before dissection. (b) Skin and abdominal muscle reflected; peritoneum intact. Considerable subcutaneous adipose tissue. (c) Peritoneal cavity opened. The stomach is filled with milk and appears white. The loops of the small intestine contain bile-stained fluid contents and appear darker. ($\times 1.7$.)

FIG. 2. Mouse (5 days old; body weight = 2.0 g.) with neonatal intestinal lipidosis. A litter mate of normal mouse in Fig. 1. (a) Before dissection. White loops of intestine clearly visible through abdominal wall. (b) Skin and abdominal muscle reflected; peritoneum intact. White loops of intestine with two 'red spots' visible through peritoneum. Scanty subcutaneous adipose tissue. (c) Peritoneal cavity opened. Abnormal loops of intestine with 'red spots' and a very small quantity of milky fluid free in the peritoneal cavity between the coils. ($\times 1.7$.)

FIG. 3. Line drawings showing the variable distribution of lesions in the small intestine of 8 abnormal mice aged 1 to 12 days. The affected parts are shaded black. The stomach is represented on the right and the large intestine on the left. (Approx. $\times \frac{1}{2}$.)

age at which this was seen was 4 days. In most of the affected animals there was no excess of fluid in the peritoneal cavity, but in a few of the most severely affected mice a minute amount of white chylous fluid was present in the peritoneal cavity (Fig. 2). This was usually not visible during life, being seen only during dissection after death. With the passage of time the abnormal china-white areas gradually became smaller and macroscopically visible lesions

were not seen later than 22 days after birth. The stomach and colon were never involved in this process and no adhesions formed in the peritoneal cavity.

If a suckling mouse is anaesthetized or killed and the mesentery is exposed, the chyle-containing mesenteric lymphatics can readily be seen with a dissecting microscope. The pattern of these lymphatic vessels varies somewhat from animal to

animal but the general arrangement and appearance seemed essentially similar in affected and unaffected animals. A significant feature was that macroscopically normal chyle-containing lymphatics could be seen leading from affected parts of the intestine. The arteries and veins in the mesentery usually appeared normal but when the blood vessels in the intestinal wall became congested after death a curious anastomosing cirroid pattern of distended blood-containing vessels appeared in the abnormal areas in some affected animals. A pattern of this kind was never seen in the normal animals.

The gastro-intestinal tract of many of the affected mice was removed *in toto* and the distribution of the lesions recorded (Fig. 3). The length of the small and large intestines was also measured and compared with those of normal mice of similar ages and origin. In these specimens the small intestine increased from an average length of about 6 cm. at 1 day to about 22 cm. at 3 weeks and the large intestine from about 2.25 cm. to about 4.25 cm. at the same ages. In this respect there were no detectable differences between normal and affected mice. In view of the profound microscopical change it is interesting that there was no obvious interference with growth of the small intestine even in animals whose weight increase was grossly retarded.

Microscopical changes. Microscopically, the principal change in the affected parts of the small intestine was an abundant accumulation of fat droplets in all layers of the intestinal wall, but particularly

in the submucosa which in well-developed examples became a very broad layer composed almost entirely of lipid (compare Fig. 5, 6, 7, and 8 with Fig. 4). The intensity and rate of development of this 'chylous oedema' varied greatly. In some animals it became severe within 24 hours, in others it took several days to reach a similar state. Often the disorder was only slight, the 'chylous oedema' diminishing fairly rapidly and the intestine returning to a normal condition within a few days without any further changes occurring. Much more frequently, however, the 'chylous oedema' persisted for a longer period and inflammatory cells began to collect in the affected parts.

If an inflammatory response occurred, it followed a fairly well-defined pattern. First, tight clusters of polymorphonuclear leucocytes appeared around capillaries or venules in the outer part of the submucosa adjacent to the main muscle coat (Fig. 8 and 9). These polymorphs never migrated far and were shortly joined by large mononuclear phagocytes which began to engulf fat. Gradually the polymorphs disintegrated and increasing numbers of macrophages, some of multinucleate giant cell form, filled the submucosa (Fig. 10, 11, and 12) and often extended into the muscle coat and subperitoneal tissue. Much of the fat was now within the cytoplasm of the macrophages and, in addition, to globular fat, crystals of lipid were sometimes found in giant cells (Fig. 13). The fat gradually diminished in amount, the macrophages became smaller and fewer, and in the majority of animals the intestine soon became structurally normal. In a few there was initially a slight increase of fibrous connective tissue in the submucosa but in older animals no definite excess of collagenous connective tissue was found. The timing and intensity of the inflammatory cellular response varied substantially from animal to animal and also from one part of the intestine to another in the same mouse. The earliest time at which clusters of polymorphs were seen in the submucosa was 2 days but even in the same animal their appearance might be delayed for about a week in portions of the intestine known to have contained abundant lipid for several days. The limitation of the polymorphs to the vicinity of the main muscle coat was a regular feature. The reason for this is not clear but it may possibly be related to some alteration in the muscle fibres of the main muscle coat. In a high proportion of these animals fat droplets were present in smooth muscle fibres of the muscle coat and in some there was undoubtedly necrosis of muscle cells. Later, bizarre enlarged nuclei were seen in certain smooth muscle cells, these resembling the regenerating smooth

FIG. 4. Jejunum of normal mouse (5 days old); a litter-mate of an affected animal. Inconspicuous submucosa and lamina propria of villi about 4 cm. from pylorus. (H. and E. $\times 50$.)

muscle fibres seen during the healing of experimentally injured arteries

Though the accumulation of lipid was usually most prominent in the submucosa, sometimes substantial amounts were also present in the lamina propria of the villi (Fig. 5, 7, and 8). With the dissecting microscope these villi usually appeared both longer and broader than normal with occasional villi having a rather bulbous form. The epithelial

cells covering these villi often contained much more lipid than is found in normal mice. Sometimes, however, the cells covering severely affected villi contained very few fat globules and were rather flattened. An interesting feature about these abnormal villi is that, despite the substantial amount of fat they contain, inflammatory cells like those found in the submucosa never seemed to collect there.

The brilliant red spots occasionally seen on the

FIG. 5. Jejunum of mouse (5 days old) with neonatal intestinal lipidosis. Moderate 'oedema' of submucosa and lamina propria of some villi: frozen sections showed abundant lipid in the submucosa and core of the villi. (H. and E. $\times 52$.)

FIG. 7. Jejunum of mouse (4 days old) with neonatal intestinal lipidosis. Abundant birefringent lipid in submucosa and in core of villi about 4.5 cm. from pylorus. From same mouse as Fig. 6. (Frozen section; polarized light $\times 38$.)

FIG. 6. Jejunum of mouse (4 days old) with neonatal intestinal lipidosis. Severe chylous oedema of the submucosa of jejunum about 4 cm. from pylorus. (H. and E. $\times 38$.)

FIG. 8. Jejunum of mouse (8 days old) with neonatal intestinal lipidosis. Severe chylous oedema of submucosa and lamina propria of villi. Clusters of inflammatory cells around small vessels adjacent to main muscle coat. (H. and E. $\times 44$.)

anti-mesenteric surface of an abnormal part of the intestine were found always to coincide with Peyer's patches. Even in newborn mice, the amount of lymphoid tissue in these patches is considerable and they normally form nodular thickenings in the intestinal wall which extend from the epithelial lining

to the main muscle coat. When fat globules accumulate in the submucosa of an abnormal portion of the intestine, the mass of lymphoid tissue in a Peyer's patch prevents much lipid collecting between it and the muscle layer external to it (Fig. 14). In affected parts of the intestine the blood vessels

FIG. 9. Jejunum of mouse (8 days old) with neonatal intestinal lipidosis. Cluster of polymorphonuclear leucocytes and some mononuclear cells in submucosa near main muscle coat. Higher power view of part of Fig. 8. (H. and E. $\times 400$.)

FIG. 10. Jejunum of mouse (12 days old) with neonatal intestinal lipidosis. Lipid-containing macrophages and some neutrophil polymorphs in submucosa. (H. and E. $\times 400$.)

within the Peyer's patches are rather distended and appear more numerous than usual, giving the lymphoid patches a bright red colour. As there is little lipid between them and the main muscle coat, the redness of the lymphoid patches is easily

visible on the peritoneal surface of the intestine. Moreover, in the living animal it is also readily seen through the thin abdominal wall.

No abnormality of the small intestine was discovered in the 11 adult mice which had suffered

FIG. 11. Duodenum of mouse (12 days old) with neonatal intestinal lipidosis. Numerous large lipid-containing macrophages in submucosa of duodenum about 1 cm. from pylorus. (H. and E. $\times 400$.)

FIG. 12. Jejunum of mouse (22 days old) with neonatal intestinal lipidosis. Several large multinucleate lipid-containing macrophages in submucosa. (H. and E. $\times 400$.)

from neonatal intestinal lipidosis. The pattern and size of the villi and the structure and cellular composition of the epithelium appeared normal. There was no detectable increase in the connective tissue content of the submucosa. The muscle coat and

the distribution and structure of the blood and lymphatic vessels appeared normal.

Other Organs and Tissues. No significant microscopical changes were discovered in the mesen-

FIG. 13. Jejunum of mouse (22 days old) with neonatal intestinal lipidosis. Crystal spaces, which had probably contained lipid, with large foamy macrophages in submucosa. (H. and E. $\times 400$.)

FIG. 14. Jejunum of mouse (4 days old) with neonatal intestinal lipidosis. Moderate chylous oedema of submucosa with a Peyer's patch. The Peyer's patch corresponds with one of the 'red spots' seen macroscopically. (H. and E. $\times 38$.)

teric lymph vessels or nodes or in any of the viscera. In those instances in which a minute amount of chylous fluid was found in the peritoneal cavity, examination of the fluid showed it to contain chylomicrons and lipid-containing macrophages.

Discussion

Comparison of the Two Disorders in Mice.

The genetic control of neonatal intestinal lipidosis has a superficial resemblance to that of chylous ascites in ragged mice. Both conditions are due to a dominant gene with imperfect penetrance (of the lymphatic condition) whose homozygote is lethal. But whereas *Nil* has no known effect on any system but the lymphatic, *Ra* affects the pelage in several ways; in addition the homozygote of the former is antenatally lethal, probably at 11 days of embryonic age, whereas the homozygote of the latter is lethal nearer birth or, in selected stocks, is occasionally viable as an adult (Slee, 1957, 1962). Penetrance of the lymphatic defect in both mutants is variable and genetically controlled, but their observed ranges of penetrance are different: from 0% to no higher than 17%, despite selection, in *Ra*+, and from 20% to 70% without selection in *Nil*+. Viability decreases with increased penetrance in *Ra*+, a feature that has not been studied in *Nil*; but whereas in *Ra*+ stocks of high and low penetrance of chylous ascites, about half of affected animals die before 4 weeks, in *Nil*+ stocks with high penetrance not more than about 10% of affected animals die before 4 weeks. The lymphatic defect in *Ra*+ is thus more severe than is the defect in *Nil*+; but in their respective homozygotes this situation is reversed.

The most striking difference lies in the genetic control of penetrance in the two mutants. From the investigation of their joint segregation, it seems that, while the *Nil* gene enhances the incidence of chylous ascites in *Ra*+, modifiers enhancing chylous ascites either depress the penetrance of *Nil* or at least fail to enhance it. The effect of *Nil* on *Ra*+ can be paralleled by that of the fidget gene, which has no known effect on the lymphatic system, but so far none of several mutants controlling other systems segregating with *Nil* appear to affect its penetrance.

The fact that enhancers of chylous ascites do not enhance the penetrance of *Nil* points to the conclusion that *Ra* and *Nil* operate developmentally in different biochemical pathways within the lymphatic system. However, there is a reciprocal situation. *Ra*+ isogenic with strain AG has a virtually zero incidence of chylous ascites (Herbertson and Wallace, 1964), and *Nil*+ isogenic with

AG has a high penetrance (66%: the first investigation described here, and unpublished). It seems therefore that modifying genes have opposite effects on the two mutants rather than merely that there are different complexes of modifiers for each. Their developmental relation must therefore be somewhat intricate.

The morbid anatomy and histology of neonatal intestinal lipidosis reflect its superficial similarity to the chylous ascites condition. In both there is a defect in the transport of fat from the small intestine after seemingly efficient digestion and absorption. In the chylous ascites mice lipid and fluid accumulate in the peritoneal cavity and also to some extent in the wall of the small intestine. But the intestinal lipidosis condition differs from the chylous ascites disorder in that lipid accumulation is almost entirely confined to the wall of the intestine and tends to affect the intestinal wall in a patchy fashion. Moreover, those parts of the intestine which are affected tend to be laden with far more lipid. The patchy involvement of the intestine and the presence of seemingly normal mesenteric lymphatic vessels without the obstructive features seen in the chylous ascites mice suggest that the defect is probably located in the wall of the intestine itself. It seems that lipid is actively absorbed from the lumen of the intestine by the epithelial cells and, instead of passing directly into lacteal vessels in the villi and leaving the intestinal wall in a normal fashion, much of the lipid is discharged into the connective tissue spaces of the intestinal wall and accumulates there. It is possible that in the affected parts the fine lymphatic channels in the intestinal wall have either not yet penetrated into the villi or, if they have, are in some other sense imperfect and therefore are unable to lead the chyle away from the intestine. Whereas in the mice with chylous ascites it seemed that there was some obstruction to lymph flow, in this second disorder it appears that faulty or inadequate development of lymphatics within the wall of affected parts of the intestine may well be responsible. However, identification of fine lymphatic vessels in conventional paraffin and frozen sections is difficult, and as yet no structural defect of these lymphatics has been discovered. Examination with the electron microscope may be much more revealing and fine structure studies are now being pursued.

Like the chylous ascites disorder, this condition appears in most of the mice to be a temporary insufficiency of lymphatic drainage occurring at a time when dramatic changes in function are being demanded. In this respect it is similar to the deficiencies of respiratory and cardiovascular function which appear at or shortly after birth when an

animal's development is either defective or as yet insufficiently mature to cope with the immense functional changes occurring at birth. From being inactive, or relatively so, the newly developed intestinal lymphatic vessels are suddenly called upon to transport a substantial volume of a watery suspension of fine fat droplets. In neonatal intestinal lipidosis it seems that the lymphatics in certain parts of the intestine are unable to fulfil this task whereas in other parts they are. As most of these animals recover completely it appears that the lymphatics either complete their development after birth or are at least modified so that they can cope with physiological needs.

The increased penetrance in genetically *Nil/+* mice following on increased milk supply is consistent with these views. It may be supposed that *Nil/+* mice which appear normal under a normal milk supply have in fact an undetected defect—a slight generalized underdevelopment or a restriction of the defect to very small areas of the intestine—and that this, under a sudden but probably short-term increase in milk supply, rapidly results in a build-up of lipid in the connective tissue spaces of the intestinal wall. Thus apparently normal mice become, under a sudden demand on the lymphatic drainage system, similar in phenotype to untreated affected *Nil/+* mice.

Lymphatic Disorders in Man and Mouse.

Various hereditary disorders of the lymphatic system, including Milroy's disease of man (Milroy, 1892) and congenital lymphatic oedema of Ayrshire calves (Donald, Deas, and Wilson, 1952) are well recognized, but there are few acceptable accounts of inherited abnormalities affecting the alimentary lymphatics of any species.

In man chylous ascites and protein-losing enteropathy may develop shortly after birth and have occasionally occurred in more than one member of a family. Though the causes and mechanisms of these uncommon disorders vary and are often obscure, abnormal development of intestinal lymphatics due to inherited defects seems to be a distinct possibility, especially when the disease begins early in life. In an investigation of the role of the gastro-intestinal system in idiopathic hypoproteinaemia, Waldmann *et al.* (1961) discovered a group of young patients in whom protein-losing enteropathy was associated with conspicuous dilatation of the lymphatic vessels of the small intestine. They suggested that the condition be called 'intestinal lymphangiectasia'. In most of these patients the dilated intestinal lymphatics contained lipid-laden macrophages and in some there were yellowish

nodules composed of similar cells along the serosal and mesenteric lymphatic vessels. In those from whom adequate specimens were available the walls of these vessels were also abnormal, showing substantial thickening and fragmentation of the elastic lamina and medial hypertrophy. All their patients had generalized oedema some time during the course of their illness, and several had chylous effusions in the peritoneal or pleural cavities. In a later investigation Pomerantz and Waldmann (1963) performed lymphangiograms on 4 patients with intestinal lymphangiectasia and discovered anomalies of the lymphatics of the lower limbs and elsewhere.

It appears, therefore, that some young people with protein-losing enteropathy have a widespread affliction of the lymphatic system. Though there are features in which these human and mouse disorders differ, they are sufficiently similar to justify a detailed comparison. Such a study might also reveal features about intestinal lymphatics which might help an understanding of certain commoner intestinal disorders, such as Crohn's disease, in which both inheritance and lymphatic obstruction may play a part.

On the other hand, these inherited conditions of mice seem to have little in common with coeliac disease and allied disorders of man. Though an inherited defect may well be a significant factor in the development of coeliac disease, the structural and functional changes in this condition appear basically different from those in the two mouse disorders. In coeliac disease villous atrophy with flattening of the epithelial cells is the characteristic lesion and is accompanied by defective absorption by the intestinal epithelium. Villous atrophy has not been seen in either of the mouse conditions and, as mentioned earlier in the discussion, the evidence favours inadequate lymphatic drainage rather than faulty absorption. Similarly, the differences between Whipple's disease ('intestinal lipodystrophy') of man and the condition described in this paper are probably much greater than their resemblances. In Whipple's disease abundant foamy macrophages are found in the intestinal wall but these abnormal cells contain conspicuous PAS-positive intracytoplasmic inclusions, and, with lesions often developing in other parts of the body, it seems likely that it is either a generalized metabolic disturbance or a bizarre infective process.

Summary

An inherited disorder in mice, due to defective transport of fat from the small intestine, is described. Shortly after the newborn mouse has

started suckling, parts of the small intestine become white. Microscopically, the abnormal portions of the intestinal wall are found to be thicker than usual and to contain abundant lipid, particularly in the submucosa. The condition often persists until after weaning.

The disorder arose as a spontaneous mutation in strain A; it is dominant, with heterozygous penetrance varying from 30 to 70% according to strain. The gene is given the symbol *Nil*, an abbreviation of the phrase 'neonatal intestinal lipidosis'.

Breeding tests demonstrated linkage of *Nil* with albinism, *c*, and pinkeyed dilution, *p*; the order and approximate recombination values are: *c*—6%—*Nil*—3%—*p*. These linkages were used to study the homozygous condition, the viability of the heterozygote, and the effect of milk supply on penetrance. The homozygote is antenatally lethal, death occurring at or before 11 days' gestation. Death-rate of the heterozygote is negligible before birth, is highest at 2–14 days (about 8%), and tails off after weaning; but adults tend to be small, sometimes infertile, and to have somewhat small litters. A sudden increase in milk supply increases penetrance.

Segregation of *Nil* with ragged, *Ra*, whose heterozygotes occasionally have chylous ascites, shows an unusual interaction. It appears that, while *Ra* has no effect on the penetrance of *Nil*, *Nil* increases the incidence of chylous ascites in *Ra*+. There are also indications that a genetic *milieu*, which increases

the incidence of lipidosis in *Nil*+, decreases the incidence of chylous ascites in *Ra*+. The basis of this interaction is discussed.

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