Chylous Ascites in Newborn Mice*

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In 1958, during breeding experiments in the Department of Genetics at Cambridge, a small proportion of newborn offspring of backcross matings of the gene ragged (*Ra*, Slee, 1957) developed a swollen belly. The affected animals appeared normal at birth but about 24 hours after they had begun to suckle their abdomens became swollen with an accumulation of milk-like fluid in the peritoneal cavity. During the first 5 days after birth the abdominal wall of normal mice is relatively transparent, and the stomach, intestines, and other viscera are clearly visible from the ventral surface.

In the affected mice, as the condition developed, milky fluid could be seen lying between the loops of the intestines (Fig. 1). In most of these animals only a small amount of fluid collected and the distension was correspondingly slight; in some, however, a larger amount collected and the viscera were partially or completely hidden (Fig. 2), the abdomen eventually becoming immensely distended.

The milky fluid was sterile and consisted principally of a fine suspension of fat in the form of innumerable minute globules but also contained some macrophages and a few other inflammatory cells. The term chylous ascites is used to describe such an accumulation of chylous fluid in the peritoneal

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FIG. 1. Mouse (3 days old) with chylous ascites (the short tail and polydactyly are due to the genes *Sd* and *py*), viewed from ventral surface. (a) Before dissection: loops of intestine and free margin of liver clearly visible through abdominal wall. (b) Skin and abdominal muscle reflected, peritoneum intact: loops of intestine contrast sharply with milky fluid. (c) Peritoneal cavity opened: milky fluid lying between loops of intestine. (All $\times 2$.)

FIG. 2. Mouse (16 days old) with chylous ascites, viewed from ventral surface. (a) Before dissection: loops of intestine visible through abdominal wall. This mouse was stunted, had very little subcutaneous fat, and hair growth was retarded. In a normal 16-day-old mouse adipose tissue would prevent the viscera being seen. (b) Skin and abdominal muscle reflected, peritoneum intact: a few coils of intestine are visible; the rest of the viscera is hidden by the milky fluid. (c) Peritoneal cavity opened: milky fluid lying between loops of intestine which, in places, were matted together. The proximal portion of the small intestine was considerably distended. Intestinal obstruction appeared to be developing. (All $\times 2.75$.)

cavity and it seemed an accurate description of this disorder.

The fate of the affected animals varied with the severity of the condition. Those in which only a small volume of fluid was present seemed just as vigorous as their healthy litter mates and gained weight normally. At about 7 days old the milky fluid was absorbed and they could not then be distinguished from their unaffected sibs. On the other hand, those in which a large amount of fluid collected were not nearly as active as the others and failed to gain weight at the usual rate. Some of these more severely affected animals recovered during the second, third, or fourth weeks of life and then developed normally. In others the amount of peritoneal fluid increased still further and at this stage they became too weak to suckle and died.

Those defective animals that survived until classification of the gene *Ra* was possible in this stock (about 7 days) were invariably found to be ragged heterozygotes (*Ra*+), i.e. to have the sparse and retarded hair growth typical of the expression of this gene. However, the majority of *Ra*+ in the colony, while capable of transmitting the abdominal defect to later generations, were not themselves

defective. Moreover, only about 1 in 10 raggeds showed chylous ascites. This suggested that the condition was due either to an imperfectly penetrating dominant closely linked to *Ra*, or to an occasional pleiotropic effect of *Ra*+

An inherited tendency to chylous ascites does not appear to have been previously recorded in any species. This account describes an investigation of its morbid anatomical and physiological features (B. M. Herbertson) and of its genetical aspects (M. E. Wallace).

Materials and Methods

Examination of Peritoneal Fluid. The peritoneal fluid from 22 mice with chylous ascites was collected *post mortem* and measured. Wet and dry preparations of the fluid were examined microscopically, and 4 samples of the fluid were cultured aerobically and anaerobically to discover whether bacteria were present.

The lipid composition of the peritoneal fluid and stomach contents of four abnormal mice was analysed and compared. The peritoneal fluid from the 4 mice was pooled and provided about 50 mg. of material; the stomach contents were similarly pooled. The two specimens were extracted with methanol:chloroform (2:1 v/v), filtered, evaporated to dryness *in vacuo*, and

then dissolved in 0.4 ml. chloroform. 0.2 ml. aliquots were investigated using thin-layer chromatography (Bowyer, Leat, Howard, and Gresham, 1963). After spraying each thin-layer plate with 2', 7'-dichlorofluorescein in ethanol, the approximate percentage and position of the lipids was evaluated visually under ultraviolet light. The fatty acids of the triglyceride fraction were then converted to their methyl esters and analysed by gas liquid chromatography.

Morbid Anatomy and Histology. Twenty-two mice with chylous ascites and 20 normal controls of both sexes and comparable ages, ranging from 1 to 27 days, were killed with chloroform and dissected shortly after death. The peritoneal fluid from the abnormal mice was collected and examined (see above). The carcasses were fixed in 4% formaldehyde saline. Transverse blocks of the abdomen and thorax at various levels were embedded in paraffin wax, and sections were stained by Ehrlich's haematoxylin and eosin and by Weigert's resorcin-fuchsin method for elastic fibres and van Gieson's mixture for collagen fibres. Other staining methods used included Mallory's phosphotungstic acid haematoxylin and McFarlane's modification of Mallory's trichrome method. Serial sections of the abdomen and thorax of 2 abnormal and 2 control mice were also prepared. In addition frozen sections of the small intestine of 9 abnormal and 7 control mice were cut and treated with a mixture of Sudan III and IV or with Sudan black. A more thorough microscopical examination of the gastro-intestinal tract of 3 abnormal and 2 control mice was made, comparable paraffin and frozen sections being prepared of the stomach, duodenum, jejunum, ileum, and colon.

Three adult mice, 8 to 12 months old, that had developed chylous ascites shortly after birth and had recovered were also killed with chloroform and examined.

Feeding Experiment. Three 2-day-old mice with chylous ascites were given a few drops of a carbon suspension (particles size not greater than 3 μ) by mouth. The passage of the carbon along the alimentary tract was observed from the ventral surface of the abdomen during the subsequent two days.

Breeding Stocks. The stock in which chylous ascites appeared in January 1958 had been formed from two closed foundation stocks. One had been segregating for 4 years for the linkage group V markers ragged (*Ra*), tan (*a*⁺), and wellhaarig (*we*). Some 1,600 mice were examined from linkage backcrosses, of which 848 were raggeds (Parsons, 1958). The number of raggeds examined in the whole experiment is probably over 1,000 when preparation matings are included. The other foundation stock had also been segregating for 4 years for these markers and for the two further linkage group V markers fidget (*fi*) and Danforth's short tail (*Sd*). It is estimated that some 1,300 raggeds were examined. The amalgamated stock segregated for a year in these 5 markers before chylous ascites was seen.

The technical assistants in charge of all these stocks examined all litters, irrespective of age, as a weekly routine, so that most young were scrutinized 2 or 3 times in the nest. Since many cases of chylous ascites are grossly abnormal, often for over a week, the chance that any cases occurred before January 1958 and were missed is extremely remote.

The new linkage group V stock was established to investigate genetical interference in the *Ra-Sd* part of the chromosome, and to this end the 16 possible types of fivefold linkage backcross were gradually made. The observations on chylous ascites were incidental to this programme, which is still under way. However, its design and the even spacing of the markers (recombination percentages are approximately *Ra-25-a⁺-12-we-22-fi-22-Sd*, Wallace, 1958) make it ideal for the discernment of any loci, whether of large or small effect on the chylous ascites phenotype, that may exist anywhere in these segments.

Indeed, it was at once apparent that the area marked by *Ra* has a major effect, for the first nine cases were all *Ra*+. No obvious correlation was found with sex or with the other 4 markers, but any weaker correlations that exist will emerge from a full analysis of the complete experiment. Meanwhile, two samples of the data, considering chylous ascites and ragged only, have been taken. The first, for 1958 to 1960, and the second, for 1961 to 1963, appear as data (a) and (b) of Table I.

It was impossible to decide which matings segregating in *Ra* but producing no chylous ascites animals were nevertheless genotypically capable of producing them. This was because affected animals nearly always came from normal parents and because the incidence of chylous ascites was low, only 1 or 2 cases occurring in a mating: initially too, affected animals seldom survived long enough to breed. However, the confinement of chylous ascites to the *Ra*+ phenotype continued. For these reasons, the data from all matings segregating in ragged, irrespective of whether or not they produced affected animals, are pooled.

Nevertheless, the possibility that affected parents were occasionally being used and that their use could alter the incidence of chylous ascites in their offspring was worth investigating. The affected animals of Table I are therefore classified in Table II according to whether their *Ra*+ parent had itself been classified as showing chylous ascites or not. Since only one parent was *Ra*+ and this could be either the father or the mother, the data are subdivided according to the sex of the *Ra*+ parent.

A small experiment on the genetic control of incidence was also started in June 1962. A single affected ragged was crossed to the standard inbred strain AG/Cam. From its progeny, two series of backcrosses of *Ra* to AG/Cam were made. In the first, only affected raggeds were used in each generation, in the second only unaffected raggeds. The former series has reached the third backcross, the latter the fifth. This process of 'grading up' is expected to make both stocks gradually isogenic with AG/Cam, theoretically complete isogenesis being reached after the fifth cross. The stocks differ

TABLE I

CLASSIFICATION OF PROGENY FROM BACKCROSSES OF *Ra*, WITHOUT SELECTION IN RESPECT OF CHYLOUS ASCITES (CHYLOUS ASCITES IS SYMBOLIZED 'Ch.As.')

Data	Non- <i>Ra</i>		<i>Ra</i>		Total	<i>Ra</i> Ch.As. Divided By All <i>Ra</i>	All <i>Ra</i> Divided By Total
	With Ch.As.	Without Ch.As.	With Ch.As.	Without Ch.As.			
(a) Jan. 1958 to July 1960	(2?)*	1248	127	1016	2391	11.1%	47.8%
(b) Oct. 1961 to Sept. 1963	0	1043	160	741	1944	17.8%	46.3%
χ^2 Analysis	Non- <i>Ra</i>		<i>Ra</i>		<i>Ra</i> With Ch.As.		<i>Ra</i> Without Ch.As.
(a) Jan. 1958 to July 1960	1250		1016		127		1016
(b) Oct. 1961 to Sept. 1963	1043		741		160		741
Contingency χ^2 Probability	4.42 < 0.05				18.4 < 0.001		

* The two possible cases of chylous ascites in non-*Ra* are doubtful because they occurred before the closeness of the association with *Ra* was realized; their classification was not, therefore, checked.

TABLE II

CLASSIFICATION OF THE PROGENY WITH CHYLOUS ASCITES (FROM TABLE I) ACCORDING TO THE CHYLOUS ASCITES PHENOTYPE OF THE *Ra* PARENT (CHYLOUS ASCITES IS SYMBOLIZED 'Ch.As.')

Data	Mother <i>Ra</i>		Father <i>Ra</i>		Total	Number From <i>Ra</i> Ch.As. Parent Divided By Total
	With Ch.As.	Without Ch.As.	With Ch.As.	Without Ch.As.		
(a) Jan. 1958 to July 1960	1	41	1	84	127	$\frac{2}{127} = 1.57\%$
(b) Oct. 1961 to Sept. 1963	2	48	16	94	160	$\frac{18}{160} = 11.25\%$

χ^2 Analysis	Parent <i>Ra</i>		Corrected contingency $\chi^2 = 8.78$ Probability = < 0.001
	With Ch.As.	Without Ch.As.	
(a) Jan. 1958 to July 1960	2	125	
(b) Oct. 1961 to Sept. 1963	18	142	

TABLE III

CLASSIFICATION OF PROGENY FROM SUCCESSIVE BACKCROSSES TO AN INBRED STRAIN, WITH SELECTION IN RESPECT OF CHYLOUS ASCITES (CHYLOUS ASCITES IS SYMBOLIZED 'Ch.As.')

Data	Non- <i>Ra</i>		<i>Ra</i>		Total	<i>Ra</i> Ch.As. Divided By All <i>Ra</i>	All <i>Ra</i> Divided By Total
	With Ch.As.	Without Ch.As.	With Ch.As.	Without Ch.As.			
(a) From <i>Ra</i> parents with chylous ascites	0	92	14	81	187	17.3%	49.2%
(b) From <i>Ra</i> parents without chylous ascites	0	46	1	49	96	2.0%	52.1%
χ^2 Analysis	Non- <i>Ra</i>		<i>Ra</i>		<i>Ra</i> with Ch.As.		<i>Ra</i> Without Ch.As.
(a) From <i>Ra</i> parents with chylous ascites	92		95		14		81
(b) From <i>Ra</i> parents without chylous ascites	46		50		1		49
Contingency χ^2 Probability	0.006 > 0.95				4.27 < 0.05

FIG. 3. Peritoneal fluid of mouse (2 days old) with chylous ascites. Macrophage (about 12μ diam.) containing droplets of fat; a red blood cell is also present. Innumerable refractile chylomicrons in the background. (Wet preparation, $\times 1,000$.)

FIG. 4. Peritoneal fluid of mouse (6 days old) with chylous ascites. A group of large macrophages (up to about 30μ diam.) containing abundant droplets of fat. (Wet preparation, $\times 1,000$.)

ultimately only for those genes selected by the choice of the ragged parent according to its phenotype in respect of chylous ascites. The data are given in Table III.

Results

Peritoneal Fluid. In mice killed during the first 16 days the peritoneal fluid was always milk-like in consistency and appearance. Later, it became progressively more 'watery', and in 2 mice killed during the fourth week the fluid was only slightly opalescent but had a curiously shimmering surface. The amount varied from a trace to 1.4 ml., but in most it was between 0.3 and 0.5 ml. Although such volumes seem small they appear as substantial amounts in the abdominal cavity of suckling mice and in some accounted for as much as 15% of the total body weight. Towards the end of the first week some fragments of a cream-coloured cheesy material were often found floating freely in the milky fluid.

Initially the most impressive microscopical

features were an abundance of minute globules of fat (chylomicrons) and some macrophages containing droplets of fat (Fig. 3). A few polymorphonuclear leucocytes and red cells were also present. The number, size, and fat content of the macrophages then gradually increased (Fig. 4), and a small number of multinucleate cells appeared. Towards the end of the first week a few colourless anisotropic crystals also began to develop. Most of these crystals were needle-like and were often clustered together in the form of tufts (Fig. 5). A few rectangular plate-like crystals also appeared. During the second and third weeks the fluid still contained abundant chylomicrons but in the fourth week more and more crystals formed, and the fatty globules became fewer. At this stage fat-containing macrophages also became less numerous. There can be little doubt that the 'milky' appearance of the fluid in the first 2 to 3 weeks was due to the abundance of chylomicrons during that period and that the opalescence and shimmering surface of the fluid

FIG. 5. Peritoneal fluid of mouse (5 days old) with chylous ascites. A tuft of needle-like crystals; a phagocyte and a few red blood cells are also present. (Wet preparation, $\times 1,000$.)

noticed particularly during the fourth week was due to the high crystal content.

No bacteria were isolated from the aerobic and anaerobic cultures of the 4 specimens of peritoneal fluid.

The results of the analysis of the lipids in the peritoneal fluid and stomach contents of 4 mice

with chylous ascites are shown in Tables IV and V. The general lipid composition of the two materials is remarkably similar, both consisting chiefly of triglycerides. The fatty acid content of the triglycerides in the peritoneal fluid and the stomach contents also corresponds within the limits of the experimental error for the method used.

TABLE IV
APPROXIMATE COMPOSITION (%) OF LIPID EXTRACTED FROM PERITONEAL FLUID AND STOMACH CONTENTS OF MICE WITH CHYLOUS ASCITES

Specimen	Triglycerides	Cholesterol	Cholesterol Esters	Free Fatty Acids	Cephalin	Lecithin	Sphingomyelin
Peritoneal fluid	70	5	5	10	Trace	5	5
Stomach contents	60	5	5	10	10	5	5

TABLE V
FATTY ACID COMPOSITION OF TRIGLYCERIDES (MOLES %) FROM PERITONEAL FLUID AND STOMACH CONTENTS OF MICE WITH CHYLOUS ASCITES

Specimen	Lauric 12:0	Myristic 14:0	Palmitic 16:0	Palmitoleic 16:1	Stearic 18:0	Oleic 18:1	Linoleic 18:2
Peritoneal fluid	3	12	32	9	1	35	5
Stomach contents	4	9	34	8	2	30	7

Note: The figure before the colon denotes the number of carbon atoms and that after it the number of double bonds.

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GENERAL. In the youngest mice the abnormalities were confined to the abdomen and consisted of the accumulation of fluid in the peritoneal cavity and changes in the small intestine and mesenteric lymph vessels. The more severely affected animals aged 2 to 4 weeks showed similar abdominal changes but in addition were smaller than their normal litter mates, and had a rather wizened appearance and a general deficiency of adipose tissue. In some of these creatures hair growth was more retarded than usual in *Ra* +.

The 3 adult mice that had developed and recovered from neonatal chylous ascites were normal apart from a few fibrous adhesions between the coils of the intestine and the other viscera.

SMALL INTESTINE. The wall of the gastro-intestinal tract of normal newborn mice is very thin and the contents of the various portions can readily be seen through it. After suckling, the stomach and proximal part of the duodenum contain milk and appear creamy white. On the other hand, the distal portion of the duodenum, the jejunum, and ileum with their bile-stained contents are usually a much deeper colour. In most of the abnormal mice the whole length of the duodenum and the proximal part of the jejunum were creamy white and the distal portion of the small intestine was unduly pale. When the gastro-intestinal tracts of the abnormal mice were opened the walls of the small intestines, particularly of the jejunal portion, were found to be thicker and paler than usual but their contents appeared normal.

Microscopically, the wall of the small intestine was abnormal in all the mice with chylous ascites. In those killed during the first 10 to 12 days the principal alteration was an accumulation of droplets of fat in the submucosa. The amount of fat varied both from animal to animal and in a particular mouse from one part of the small intestine to another. In a few mice only a small quantity of fat was present but in others there was a substantial amount, and the submucosa, normally an insignificant structure, formed a broad layer bloated with fat (Fig. 6, 7, 8, and 9). The largest amount of fat was always to be found in the jejunum but even in the least affected animals fat was usually also present in the submucosa of the duodenum and the ileum. At first most of the fat was in the form of droplets but towards the end of the first week acicular crystals began to appear. In frozen sections much of the fat was anisotropic (Fig. 10) and some showed 'maltese-cross' birefringence. There was remarkably little cellular response to the presence of the

FIG. 6. Small intestine of mouse (3 days old) with chylous ascites. Broad 'oedematous' submucosa: frozen sections showed that the 'oedematous' appearance was due to the presence of fat. (H. and E. $\times 52$.)

FIG. 7. Small intestine, abdominal wall, and part of stomach of normal mouse (3 days old). The submucosa of the normal small intestine is an insignificant layer. (H. and E. $\times 52$.)

FIG. 8. Jejunum of mouse (5 days old) with chylous ascites. 'Oedematous' appearance of submucosa of jejunum about 3.5 cm. from pylorus. The diameter of the jejunum is greater than normal. (H. and E. $\times 52$.)

FIG. 10. Jejunum of mouse (5 days old) with chylous ascites. Birefringent lipid in submucosa and in core of villi of jejunum about 4 cm. from pylorus. From same mouse as Fig. 8. (Polarized light, $\times 52$.)

FIG. 9. Jejunum of normal mouse (5 days old). Insignificant submucosa of jejunum about 3.5 cm. from pylorus. (H. and E. $\times 52$.)

FIG. 11. Jejunum of mouse (13 days old) with chylous ascites. Wide sinusoidal channels containing blood in submucosa. (H. and E. $\times 52$.)

fat in the submucosa and although some was engulfed by macrophages most was lying freely. A few small clusters of polymorphonuclear leucocytes were also occasionally present in the submucosa.

Although the accumulation of fat was always much more conspicuous in the submucosa than in the other layers of the intestinal wall, the core of the villi also often contained more fat than usual. In addition, small droplets of fat were frequently present in smooth muscle cells, particularly those in the inner portion of the circular muscle layer adjoining the submucosa. In contrast, no abnormality was discovered in the epithelial cells lining the small intestine. The cells were of normal size, shape, and distribution, and they contained a normal amount of fat.

Towards the end of the second week the amount of fat in the wall of the small intestine rapidly diminished, and during the third and fourth weeks only very small quantities remained. At this stage the most notable change was the presence in the submucosa of widely distended sinusoidal channels containing blood (Fig. 11).

MESENTERIC LYMPH VESSELS AND NODES. If the mesentery of the small intestine of a suckling mouse is gently spread out the mesenteric lymph vessels filled with chyle can readily be seen coursing from the intestine towards the root of the mesentery. A particularly good view of these vessels can be obtained by using a dissecting microscope. In 5 mice with chylous ascites killed during the first week the mesenteric lymph vessels appeared unduly distended and tortuous, and milky fluid could be seen oozing from the surface of the mesentery and also from the peritoneal surface of the jejunum. This alteration was seldom observed in abnormal mice killed during the second and subsequent weeks. Moreover, the distension of the lymph vessels observed during the first week was never as prominent in paraffin sections as it had seemed during necropsy.

The size and number of lymph nodes at the root of the mesentery seemed to be similar in both the affected and normal mice, but in those with chylous ascites the sinuses in some of the mesenteric nodes were uniformly filled with blood, and a few megakaryocytes were often present among the lymphocytes in the denser lymphatic tissue. These unusual nodes were probably haemal nodes or haemal lymph nodes. None of the nodes showed any evidence of infection or neoplasia.

PERITONEUM. Flakes of cream-coloured cheesy material began to form on the peritoneal surface of the viscera towards the end of the first week. This

material was particularly prominent in the neighbourhood of the liver and pancreas and consisted of lipid-filled cells, droplets of fat, acicular and plate-like crystals, and cell debris closely packed together (Fig. 12 and 13). Most of the cells were macrophages, some being multinucleate, but a few polymorphonuclear leucocytes were also present.

At first, this material was only loosely attached to the peritoneum, but during the second and third weeks it became more firmly adherent and in severely affected animals the loops of intestine and other viscera became matted together. During this period the number of recognizable cells diminished and the material tended to resemble the pultaceous mush seen in human atherosclerosis. In places it became invaded by granulation tissue and the fibrous adhesions seen in the 3 adult mice were probably the end stage of this process.

LIVER. In most of the more severely affected animals the liver cells immediately beneath Glisson's capsule contained abundant fat in their cytoplasm (Fig. 14). The rest of the liver appeared normal and the haemopoietic tissue present at birth diminished at about the same rate in both affected and normal animals.

Feeding Experiment. The carbon suspension fed to the three 2-day-old mice blackened the contents of the stomach and intestines but none escaped into the peritoneal cavity. When viewed through the ventral wall of the abdomen the black loops of the intestine contrasted sharply with the white chylous fluid.

Genetical Data

CONFINEMENT OF CHYLOUS ASCITES TO *Ra+*. In both samples of the linkage group V stock, cases of chylous ascites remained confined to ragged heterozygotes, only 2 doubtful cases in non-raggeds being recorded (Table I). In the small isogenesis experiment also, only raggeds were affected (Table III).

CHANGES IN INCIDENCE OF CHYLOUS ASCITES IN *Ra+*. In the 1958-60 sample, only 2/127 raggeds with chylous ascites came from a similarly affected parent (Table II), but in the 1961-63 sample the proportion rose to 18/160 (Table II). The difference is significant ($\chi^2 = 8.78$ for 1 d.f., probability less than 0.001).

The incidence of chylous ascites in the ragged progeny also showed an increase, namely from 11.1% in the earlier data to 17.8% in the later (Table I). This increase is even more significant ($\chi^2 = 18.4$ for 1 d.f., probability much less than 0.001).

FIG. 12. Liver and diaphragm of mouse (10 days old) with chylous ascites. An accumulation of lipid-containing macrophages and some polymorphonuclear leucocytes in peritoneal cavity between diaphragm (above) and liver. (H. and E. $\times 100$.)

FIG. 13. Pancreas of mouse (13 days old) with chylous ascites. Collection of lipid-containing macrophages and other cells in peritoneal cavity adjoining pancreas. (H. and E. $\times 400$.)

FIG. 14. Liver of mouse (6 days old) with chylous ascites. The liver cells immediately beneath Glisson's capsule are enlarged and vacuolated. Frozen sections showed that these cells contained abundant fat. Normal amount of haemopoietic tissue for age. (H. and E. $\times 100$.)

In the isogenesis experiment which started with a single affected ragged, the series in which only affected raggeds were used in each generation has an over-all incidence of 17.3% (Table III). This is close to the incidence of the stock from which it was derived, namely 17.8%. In the series in which only unaffected raggeds were used (after the first affected one), the over-all incidence is much lower, 2.0% (Table III). The difference is just significant ($\chi^2 = 4.27$ for 1 d.f., probability less than 0.05).

CHANGES IN VIABILITY OF RAGGED. The increase in incidence of chylous ascites in raggeds from the two samples of the linkage group V stock was accompanied by a slight decrease in viability of *Ra+*. From 47.8% of all progeny, a frequency close to the expected 50% for full viability, the proportion of raggeds fell to 46.3% (Table I); the difference is just significant ($\chi^2 = 4.42$ for 1 d.f., probability less than 0.05). Clearly this is a smaller change than the change in incidence.

In the isogenesis experiment, the greater incidence in the selected series over that in the unselected is also accompanied by a difference in the viability of ragged (Table III). It is again in the same direction as in the larger data, but it is insignificant ($\chi^2 = 0.006$ for 1 d.f., probability greater than 0.95); in fact the proportion of raggeds is in both cases close to the 50% expectation for full viability.

Discussion

Morbid Anatomical and Physiological Aspects. Milky fluid of various kinds may collect in the peritoneal cavity of man and other animals. In chylous ascites the fluid consists of chyle that has leaked from lymphatic vessels into the peritoneal cavity as a result of rupture or obstruction of the thoracic duct or its main abdominal tributaries. In man the most frequent causes of this condition are injury, tuberculosis, filariasis, and malignant tumours involving the lymphatic system. Apart from true chylous ascites, fluid with a milky appearance may be found in certain other circumstances. For example, a chronic inflammatory effusion in a serous cavity sometimes becomes milky due, it is claimed, to the liberation of fat from disintegrating inflammatory cells. In the present context it is also conceivable that in newborn animals a substantial developmental defect of the stomach or duodenum might allow milky liquid to escape directly into the peritoneal cavity.

If all aspects of the disorder in the young mice are taken into account there can be little doubt that the

condition is a true chylous ascites due to defective lymphatic drainage. The features favouring this view are the close time relation between the onset of suckling and the appearance of fluid in the abdominal cavity, the excessive distension of the mesenteric lymph vessels, the oozing of milky liquid from the mesentery, and the chylous nature of the fluid. On the other hand, the natural history of the disorder and the composition of the fluid provide no support for the idea that a chronic inflammatory process is the fundamental abnormality. Moreover, the failure during dissection to find any gross communication between the gastro-intestinal tract and the peritoneal cavity makes it most unlikely that the milky fluid consists of milk and digestive juices that have leaked through a developmental fault in the wall of the alimentary tract. This opinion is supported by the results of the carbon feeding experiment. In addition, if a mechanism of this kind were responsible, the affected mice would almost certainly have developed acute peritonitis, and this was never observed.

Although inadequate lymphatic drainage appears to be the likely mechanism of this condition, a convincing anatomical explanation for this deficiency has not been satisfactorily demonstrated. The absence of chylothorax and lymphatic oedema of the trunk and extremities suggests that the defect is limited to the abdominal lymph nodes and vessels and the frequent recovery of affected mice indicates that obstruction to the passage of chyle is often a temporary phenomenon. Examination of the lymph nodes and main lymphatic ducts in the abnormal animals revealed no evidence of an inflammatory or neoplastic disorder that might account for the obstruction. However, some of the mesenteric nodes had blood in all or most of their sinuses and appeared to be haemal or haemal lymph nodes rather than true lymph nodes. If this interpretation is correct it is conceivable that unusual communications between blood vessels and lymphatic channels had formed in this region during foetal life and, by converting potential lymph nodes into haemal nodes, had reduced effective lymphatic drainage. Although this idea may be incorrect it is likely that in the affected animals this portion of the lymphatic system is inadequately developed at the time of birth and is unable to cope with the substantial demands suddenly placed upon it when suckling begins. In other words, this disorder may be counterpart in the lymphatic system of the various hypoplastic conditions of other systems that cause functional embarrassment at or after birth.

There has been much controversy about various aspects of fat absorption including the route be-

which fat is transported from the small intestine. For many years after the classical study of Munk and Rosenstein (1891) it was believed that about 60% of absorbed fat passed from the intestinal epithelium into the lacteals, the remaining 40% going directly into the blood-stream. More recently, however, Bloom, Chaikoff, Reinhardt, Entenman, and Dauben (1950), Bloom, Chaikoff, Reinhardt, and Dauben (1951), and others have provided convincing evidence that in normal circumstances after feeding common fats, such as the triglycerides of the longer chain fatty acids, practically all the fat absorbed from the intestine enters the lymphatics with very little passing into the intestinal blood capillaries. Although investigation of the abnormal mice yields no information about the actual amounts of the various lipids entering the lymphatics or blood capillaries the similarity of the lipid composition of the peritoneal fluid and of the milk taken from the stomach is impressive.

In an investigation of the genetics, morphology, and development of ragged, Slee (1957) found that a high proportion of *Ra Ra* mice developed generalized oedema in the embryo stage and died either *in utero* or shortly after birth. Of the various possible causes of this oedema, Slee considered that heart failure or an endocrine abnormality were the most likely. However, there appear to be no features in his account that refute the possibility of its being due to a fault in lymphatic drainage. Indeed, the generalized oedema and the chylous ascites may both be an expression of lymphatic insufficiency, the differences between them resulting from variations in the severity and position of the defects.

Genetical Aspects. It is clear from all the genetical data that the incidence of chylous ascites could not have been maintained at 11–17% unless *Ra*, or a dominant close to it, were the main factors responsible. A major factor independent of *Ra*, or several independent factors with cumulative effect, could not alone maintain this incidence in the face of 5 years (5–20 generations) of natural selection against a somewhat deleterious phenotype and 3 crosses to a stock in which chylous ascites has never been seen.

In the latter experiment a normal linkage group V chromosome is supplied in each generation. Chylous ascites must therefore be due to a dominant: either to an imperfectly penetrating dominant closely linked with *Ra*, or to an occasional pleiotropic effect of the dominant *Ra* itself.

Imperfect penetrance often has an environmental

component. However, a genetic component is strongly indicated when the penetrance varies according to the direction of genetic selection enforced. This is the case here. For, in the linkage group V stock, an increased use of affected parents was accompanied by an increased incidence of chylous ascites in ragged progeny; while in the isogenesis experiment the series in which only affected parents were used in each generation had a greater incidence of the condition in the ragged progeny than in the series where only unaffected parents were used.

The present analysis does not disclose where these modifying loci are situated. But there is a suggestion that part of the *Ra-Sd* segment itself may contain one or more of them; in the isogenesis experiment where the incidence is 17%, this whole segment, which came from the linkage group V stock, has survived intact; whereas in the series with 2% incidence, only the *Ra-a*⁺ segment has been maintained. This possibility can be further tested in a fuller analysis of the linkage group V stock where the 5 markers of this segment segregate simultaneously in all combinations.

It seems very likely that modification of viability of the chylous ascites phenotype has accompanied its increase in penetrance. In both sets of data the change or difference in penetrance has been greater than the change or difference in ragged viability: indeed in the isogenesis experiment where the incidence is 17% there is no loss of raggeds at all, and the condition of the chylous ascites cases has not been extreme. In effect, selection operates so that as more raggeds express the condition this expression is such that its deleterious effects are lessened. This course of events, predictable from the nature of selective forces, has probably not been demonstrated before, owing to the lack in other genetic situations of a fully penetrant gene identifying the genotype of the imperfectly penetrant factor.

It was hoped that the present work might discriminate between the hypotheses of pleiotropy of *Ra* and of adjacent loci. However, as long as penetrance of chylous ascites is imperfect, only the occurrence of chylous ascites in a non-ragged would give the preliminary evidence needed for the possibility of crossing-over. In the present work only 2 doubtful instances have been reported out of some 300 cases of chylous ascites examined.

Nevertheless, there are other considerations that make one hypothesis more plausible than the other. The first is the sudden appearance of chylous ascites in the Cambridge stocks. As mentioned earlier, until January 1958 at least 2,300 raggeds

were rigorously examined in the nest, and none showed chylous ascites. In the 1,143 raggeds examined in the 2 years after that date, the incidence is 11%, a sudden and very significant increase. The second is the fact that Slee (1957, 1962) does not mention cases with milky fluid in the peritoneum and indeed has not seen the condition in any of some 5,000 or more ragged heterozygotes that he has reared (personal communication).

It seems highly likely that a spontaneous mutation occurred in 1958. Since all the ragged stocks in the Genetics Department show the same hair defect and the same generalized oedema in homozygotes as do those in Dr Slee's hands, the supposition of a separate locus to account for the chylous ascites condition is very plausible. The symbol *Chy* is proposed.

A third consideration is the virtual or complete absence of chylous ascites in Dr Slee's ragged homozygotes. In general, the homozygotes of an imperfectly penetrating mutant show a very much higher incidence and expression of the defects common in the heterozygotes, and selection capable of reducing the penetrance in heterozygotes to zero is usually insufficient to reduce the penetrance in homozygotes to anything like the same extent. In unselected stocks ragged homozygotes die before suckling starts and so cannot show chylous ascites. In Dr Slee's selected stocks, where *Ra Ra* were viable until after suckling, it is to be expected that, if *Ra* itself causes chylous ascites, the incidence of the condition, zero in the heterozygotes, might be low in homozygotes but hardly zero. However, this is the case. Of some 2,500 homozygotes, only 4 (i.e. less than 0.2%) showed anything that can be interpreted as chylous ascites, namely milky fluid in the peritoneum, and these were at that time thought to be cases of ruptured intestines (personal communication). On the other hand, absence of chylous ascites in *Ra Ra* is wholly to be expected if a second mutant, *Chy*, controls the condition, and was absent in Dr Slee's stocks.

Even if these 4 cases were indeed affected with chylous ascites, the hypothesis of a separate locus for *Chy* remains very plausible. It has been pointed out above that the oedema of *Ra Ra* could be due to a defect in the lymphatic system, and that chylous ascites certainly is due to a fault in lymphatic drainage. A very low incidence of chylous ascites in *Ra Ra* without *Chy* would not be unexpected if the fault causing oedema were itself an enhancer of *Chy*. The situation has analogies in other genetic systems. For example, sporadic cases of polydactyly have occurred in a number of stocks of mice. Selection of the residual genotype and of mutants at

the pallid and fidget loci can, however, in the presence of the gene *py* homozygously, raise its incidence to 100%; if this stock is now made homozygous non-*py*, a low incidence of polydactyly remains (Bodmer, 1960). It is thus possible that the *Ra* locus with its oedematous effect is in the same relation to the gene *Chy* as are the selected residual genotype, and pallid and fidget mutants, to the gene *py*; namely, each can produce a high incidence of the defect in the presence of the gene concerned, and a low one in its absence.

If *Ra* is an enhancer of *Chy* or even necessary for its expression, then the chance of finding the latter in non-*Ra* is indeed low. Further genetic investigation, along with further study of correlated defects of the lymphatic system, is clearly needed for final discrimination between pleiotropy of *Ra* and a separate locus *Chy*. Meanwhile, on the current evidence, the use of the symbol *Chy* appears both justified and convenient.

Summary

Chylous ascites has been observed in 11 to 17% of newborn heterozygous ragged (*Ra*+) mice in a Cambridge stock. The affected mice appeared normal at birth but, after suckling, milky fluid gradually accumulated in the peritoneal cavity. The fluid was sterile, contained innumerable chylomicrons, and in its lipid composition resembled milk. The wall of the small intestine was much paler and thicker than usual, its submucosa being bloated with droplets of fat. There was no gross communication between the lumen of the alimentary tract and the peritoneal cavity. The mesenteric lymph vessels were distended and tortuous and some of the mesenteric nodes appeared to be haemal nodes rather than lymph nodes. There was no evidence of infection or neoplasia. Many of the mice recovered completely but the more severely affected became too weak to suckle and died. The disorder is believed to be a true chylous ascites due to inadequate lymphatic drainage from the small intestine, but an anatomical explanation for the insufficiency has not yet been fully established.

On data for *Ra* from Cambridge and Edinburgh it appears that while an occasional pleiotropic effect of *Ra* cannot be completely excluded this inherited tendency to chylous ascites is probably due to a closely linked dominant. The symbol *Chy* is proposed. As penetrance of *Chy* increases, its deleterious effects decrease. There is a possibility that *Ra* enhances penetrance and that there are other modifiers in linkage group V.

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