Tests for direct neutralizing and toxic effects of 6-AN on vaccinia virus proved negative; this implies that its action is directed against intracellular synthesis of virus.

Note added in proof. Since the submission of this letter for publication, S. H. S. Lee (*J. Bact.*, 88, 885; 1964) has reported similar results.

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Haematological Changes in Viral (*MHV*-3) Murine Hepatitis

In earlier work we investigated some biochemical[1] and biological[2] aspects of hepatitis induced in mice by mouse hepatitis virus strain 3 (*MHV*-3)

In the present communication, in order to investigate extrahepatic pathology due to *MHV*-3 (ref. 3), the peripheral blood as well as the lymphopoietic and haemopoietic systems were examined during various stages of the disease. Moreover, the virus concentration in the peripheral blood, bone marrow, spleen and lymph nodes was determined.

Eighty mice of the *NMRI*-strain, weighing about 12 g and fed on the 'Altromin' diet, were divided into four groups of 20 animals each, indicated as *A*, *B*, *C* and *D*. Mice of groups *A*, *B* and *C* were inoculated intraperitoneally with 100 LD_{50} of *MHV*-3 virus; mice of group *D* served as controls.

The number of red cells, white cells, platelets and reticulocytes in the peripheral blood was determined for eight animals from each group, picked at random among the survivors at 24 h (group *A*), 48 h (group *B*) and 72 h (group *C*). Also determined were the haemoglobin concentration and differential white cell count.

Five animals from each group were then killed by decapitation; the bone marrow was taken from the femoral diaphysis of each mouse; smears were prepared and the myelogram determined. Furthermore, from the animals killed 72 h after virus inoculation (group *C*), samples of blood, bone marrow, lymph nodes and spleen were obtained and assayed for viral content (the LD_{50} was determined according to the method of Reed and Muench[4]). From the spleen and lymph nodes of these animals histological preparations were also made.

The results summarized in Table 1 show a statistically significant drop in haemoglobin, white cells, reticulocytes and platelets. The diminution progresses with time, being particularly conspicuous in the terminal stage of the disease (72 h), when marked anisocytosis and poikilocytosis of erythrocytes also occur. The differential count, starting at 48 h, shows a marked absolute diminution of lymphocytes and, to a lesser degree, of monocytes and eosinophilic polymorphonuclear leucocytes. These alterations, which become more evident as the disease progresses, are statistically significant. 72 h following virus inoculation, absolute neutropenia is detectable. Examination of smears reveals that granulocytes are mainly represented by juvenile elements with little segmentation of the nuclei.

Table 2 shows a diminution in the elements of the erythropoietic series as compared with the leucopoietic series. The leucopoietic series shows a marked relative increase of the more immature elements (myeloblasts and promyelocytes) compared with the more mature ones (metamyelocytes and granulocytes). Furthermore, a relative increase of haemocytoblasts is present.

A histological examination of axillary lymph nodes showed a marked diffuse hyperplasia of the reticuloendothelium, with subsequent obliteration of sinuses of the medulla and dissociation of follicles of the cortex. The follicles displayed a high degree of hyperplasia, being sometimes reduced to a small clump of lymphoid elements at the periphery of the lymph node. The lymphoid cells showed frequent karyorrhexis and karyolysis. Marked hyperplasia of the reticuloendothelium of peripheral sinuses also occurred. The endothelium of sinuses of the medulla was hypertrophic and hyperplastic, with packing of the endothelial cells which often resembled multinucleated giant cells. Mitoses were often seen in reticular cells. Similar changes were detected in mesenteric lymph nodes.

The histological examination of the spleen showed a high degree of congestion of oedema. In the cortex there was a marked decrease in follicle volume. The follicular remains were made up mainly of cells of the lymphoblastic and histiocyte type, often nucleolated and in mitosis. Therefore, there were few mature lymphoid elements concentrated in a narrow band at the periphery of the follicles or scattered throughout the parenchyma. In the germinal centres there were frequent cellular changes with fragmentation of the nuclear membrane and chromatolysis. Scattered areas of necrosis were also evident.

The results summarized in Table 3 show that the virus is present in high concentration in the spleen and bone marrow, less in the peripheral blood and lymph nodes.

Table 1. Values of Haemoglobin, Red Blood Cells, Leucocytes and Platelets in Normal Mice and in Mice infected with 100 LD_{50} of *MHV*-3 Virus, at Different Times following Inoculation

	Normal mice	Infected mice					
		24 h		48 h		72 h	
Cells/mm³	Mean values* ± *S.D.*	Mean values* ± *S.D.*	*P*	Mean values* ± *S.D.*	*P*	Mean values* ± *S.D.*	*P*
Haemoglobin (g%)	14 ± 0·8	13 ± 0·6	< 0·05	13 ± 0·8	< 0·05	12 ± 0·7	< 0·01
Erythrocytes	9,612,500 ± 229,850	7,787,500 ± 208,010	< 0·001	7,271,875 ± 150,450	< 0·001	6,781,250 ± 991,100	< 0·001
Reticulocytes	120 ± 15·0	86 ± 6·9	< 0·001	17 ± 6·2	< 0·001	14 ± 3·2	< 0·001
White cells	6,937 ± 1,545	4,562 ± 1,050	< 0·001	3,031 ± 633	< 0·001	2,562 ± 311	< 0·001
Neutrophils	2,119 ± 515·9 (30·55%)	2,645 ± 939·0 (57·98%)	> 0·05	1,739 ± 479·7 (57·37%)	> 0·05	1,530 ± 259·9 (59·72%)	< 0·05
Eosinophils	109 ± 88·6 (1·57%)	34 ± 17·7 (0·74%)	< 0·05	18 ± 9·0 (0·59%)	< 0·05	11 ± 10·3 (0·43%)	< 0·05
Basophils	19 ± 20·2 (0·27%)	9 ± 11·0 (0·20%)	> 0·05	6 ± 5·1 (0·20%)	> 0·05	4 ± 5·4 (0·16%)	> 0·05
Lymphocytes	4,421 ± 881·6 (63·73%)	1,759 ± 402·8 (38·56%)	< 0·001	1,189 ± 244·3 (39·23%)	< 0·001	975 ± 176·4 (38·06%)	< 0·001
Monocytes	269 ± 153·6 (3·88%)	115 ± 54·1 (2·52%)	< 0·05	79 ± 49·9 (2·51%)	< 0·01	42 ± 24·8 (1·64%)	< 0·01
Platelets	1,012,000 ± 18,420	657,000 ± 23,790	< 0·001	460,000 ± 22,600	< 0·001	373,000 ± 16,030	< 0·001

* From eight determinations, each on a different animal.
For cell counts and haemoglobin determination the tail venous blood was used. Differential white cell counts were made on 500 elements.

Table 2. BONE MARROW PICTURE IN NORMAL MICE AND IN MICE INFECTED WITH 100 LD_{50} OF *MHV*-3 VIRUS AND KILLED AT 72 H

Per cent	Normal mice Mean values* ± S.D.	Infected mice Mean values* ± S.D.	P
Haemocytoblasts	1·36 ± 0·59	3·38 ± 0·81	< 0·01
Proerythroblasts	1·44 ± 0·26	1·14 ± 0·63	> 0·05
Basophilic erythroblasts	13·22 ± 1·60	5·16 ± 0·64	< 0·001
Polychromatic erythroblasts	3·83 ± 0·81	2·56 ± 0·69	> 0·05
Orthochromatic erythroblasts	5·12 ± 1·09	3·38 ± 0·67	< 0·05
Myeloblasts	5·78 ± 1·00	25·40 ± 2·06	< 0·001
Promyelocytes	5·69 ± 0·84	15·30 ± 1·69	< 0·001
Myelocytes	15·54 ± 1·46	16·22 ± 1·49	> 0·05
Metamyelocytes	21·30 ± 1·64	13·24 ± 1·96	< 0·001
Granulocytes	23·46 ± 1·45	12·46 ± 1·98	< 0·001
Megakaryocytes	0·40 ± 0·46	0·34 ± 0·11	> 0·05
Lymphocytes	1·32 ± 0·44	0·74 ± 0·33	< 0·05
Monocytes	0·68 ± 0·28	0·40 ± 0·22	> 0·05
Plasmacytes	0·39 ± 0·19	0·24 ± 0·11	> 0·05

* From five determinations each on a different animal. In each smear 1,000 cells were counted (May-Grünwald-Giemsa staining).

The peroxidase reaction was used to differentiate haemocytoblasts from myeloblasts. Only the 72-h values are reported in the table, as they were the most significant.

Table 3. VIRUS CONCENTRATION IN PERIPHERAL BLOOD, BONE MARROW, SPLEEN AND AXILLARY LYMPH NODES OF MICE INFECTED WITH 100 LD_{50} OF *MHV*-3 VIRUS 72 H PREVIOUSLY

Materials	LD_{50}
Blood	$10^{-5.25}$
Bone marrow	$10^{-6.15}$
Spleen	$10^{-6.50}$
Axillary lymph nodes	$10^{-4.15}$

From the results presented here, the following conclusions can be drawn: (1) The hypoplasia of the erythroid series detected in the bone marrow, and particularly the considerable pathological changes of these elements, might be responsible for the marked and progressive anaemia, reticulocytopenia and diminution of haemoglobin noted in the peripheral blood. (2) The marked maturative delay and pathological changes observed in the myelopoietic series of bone marrow and the histological damage present in cells of the lymphopoietic regions of the organism (spleen and lymph nodes) might account for the leucopenia. (3) The alteration of the maturative process of megakaryocytes in the bone marrow might explain the marked thrombopenia of the peripheral blood.

The high virus concentration in the bone marrow and lymphopoietic tissues suggests that the aforementioned changes might be produced by an active multiplication of the virus in these organs.

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GENETICS

Inheritance of Self-incompatibility and Brown Keel Tip in *Lotus corniculatus* L.

Lotus corniculatus is a segmental allotetraploid[1,2] which frequently exhibits chromosomal tetrasomic inheritance[3–7]. We have investigated the inheritance of pubescence, a chlorophyll deficiency, cyanogenesis, flower colour, streaks on the corolla, keel tip colour and self-incompatibility. We obtained evidence for chromosomal tetrasomic inheritance with each characteristic studied. However, we also found a number of crosses which do not give good fits to those expected with tetrasomic inheritance and feel these warrant further study.

Our most extensive investigation is of self-incompatibility, a continuation of work described previously[8]. We developed a technique, based on that of Picard and Demarly[9], that permits us to distinguish between compatible and incompatible matings. This technique involves growing pollen on an agar medium which is pretreated with stylar extracts. Two plant families were studied. One consisted of 19 S_1 progeny and their relatively self-fertile parent. These were tested in all possible combinations. The other consisted of cross-progeny of two unrelated plants. Two cross-progenies of eight plants each (the one reciprocal to the other) were tested in all possible combinations. The self-progeny group of plants gave a reasonably good fit to the pattern expected if self-incompatibility is determined by *S*-alleles inherited tetrasomically at a single locus. The two cross-progenies gave reasonably good fits to the pattern expected if self-incompatibility is determined by *S*-alleles inherited disomically at two loci. If we allow for the two different segregation patterns in the two families, our data accord with the hypothesis that we are dealing with a *Nicotiana*-type gametophytic oppositional incompatibility system. We have to allow for compatibility due to dominance in the pollen grain. When a recessive S-allele present in the pollen is matched in the style but a dominant allele in the same pollen grain is not matched, the pollen is compatible.

These studies suggest the following hypothesis on which further studies may be based. The four chromosomes carrying the *S*-alleles are homologous in the self-progeny, so we get random pairing by twos to give chromosomal tetrasomic inheritance. The four chromosomes carrying the *S*-alleles in the cross-progeny consist of two structurally differentiated pairs to give disomic segregations at two loci. If this is true, we should be able to find plants which will produce progeny that segregate tetrasomically in some crosses and disomically in others, and there should be intermediate segregations.

This hypothesis should also be tested with other segregating characters. A procedure that may be used is to cross a quadruplex with a nulliplex plant and examine segregations after back-crossing to the nulliplex. If there is a tendency for the two chromosomes from one parent to pair together (that is, two carrying the dominant allele), there should be an increase in simplex progeny compared to the 1 duplex : 4 simplex : 1 nulliplex expected with random chromosomal tetrasomic inheritance. Conversely, a tendency for one chromosome from one parent to pair with one from the other should result in a decrease in simplex progeny. The progeny genotypes may range from all simplex to 1 duplex : 2 simplex : 1 nulliplex, and expected phenotypic ratios for complete dominance may range from all the dominant phenotype to 3 dominant : 1 recessive. These extremes correspond to ratios expected with disomic inheritance at two loci. If we get the extreme condition and one pair of chromosomes is homozygous and the other heterozygous at this locus, we would expect simple disomic segregation ratios. However, a small degree of tetrasomic pairing would be expected to break down disomic patterns. If this hypothesis were valid, we would expect duplex siblings to differ from each other when back-crossed to the same recessive parent. Also, we must allow for the possibility that the degree of preferential pairing may be influenced by environmental effects so that test-crosses made at different times may segregate differently. On the other hand, test-crosses made to different nulliplex plants at one time should not differ significantly.

It is obviously very difficult to test an hypothesis as flexible as this. It is possible to reconcile most of the data we have obtained so far with this hypothesis. Other published data, such as that of Buzzell and Wilsie[5], also appear to fit this hypothesis. It accounts for the excess of brown keel tip they obtained when they backcrossed duplex with nulliplex plants. It does not account for the deficiency of brown keel tip they observed with simplex crosses. However, we have evidence that supports their postulate that another locus is segregating restraint or