"t" was calculated and P determined to be less than 0.001.

Table I summarizes the data obtained for MD activity in the tissue homogenates. The activity of this enzyme in human colonic adenocarcinoma and uninvolved tissue was within the same range as that observed for LD. MD activity in 16 tumor homogenates varied from 227 to 1760 units with an average activity of 1001 units, while the activity in the 16 samples of adjacent tissue from the organ involved varied from 97 to 909 units with a mean activity of 468 units. In 15 of the 16 sets of tissue samples assayed the activity of MD in the carcinoma was higher than that in the uninvolved tissue. The difference between the MD activity in the tumor and in the neighboring cells is significant at the 0.001 level.

The data obtained for enolase are also summarized in Table I. In homogenates of both adenocarcinoma of the colon and adjacent intestinal mucosa the activity of enolase was much lower than the activities of both LD and MD in the uninvolved tissues. The activity of this enzyme varied from 15 to 158 units with an average activity of 78 units in the homogenates of the malignant tissue samples. Enolase activity in the uninvolved colon tissue homogenates varied from 7 to 76 units with a mean activity of 38 units. In the tissues of 13 of the 16 patients studied, the activity of enolase was higher in the neoplastic tissue than in the uninvolved tissue. The difference in enzyme activity between the 2 groups is significant at the 0.001 level.

Summary. Homogenates of the neoplastic and adjacent non-malignant tissues from patients with adenocarcinoma of the colon were prepared and assayed spectrophotometrically for LD, MD, and enolase. Significant differences in enzyme activity were observed between the tumor homogenates and the homogenates of the uninvolved tissue. The activities of LD, MD, and enolase were significantly higher in the tumor preparations than in the preparations of adjacent mucosal tissue. These results are consistent with the hypothesis that the tumor proper is one of the sources of the elevated serum levels of the 3 enzymes.

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Identification of WM1 as LDH Virus, and Its Recovery from Wild Mice in Maryland. (29436)

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Wild house mice in Queensland, Australia, were found commonly to be infected with an obscure mouse-pathogenic agent (WM1) which could be recognized only by production of low grade chronic splenic hyperplasia (1,2). The infectious process in experimental mice was unique in several respects, namely, the lifelong persistence of virus in tissues and serum at titers of about 10^4 ID_{50} per gram, and the lack of antigenicity for mice, rats, guinea pigs, and rabbits. WM1 virus is ether-sensitive and heat labile (56°C 30 min).

Since these various characteristics closely resembled those of the lactic dehydrogenase

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(LDH) virus of Riley *et al*(3,4), studies were done to determine the possible identity of WM1 and LDH viruses. In the absence of specific immunologic tests for either virus, reliance was placed on comparison of their biologic properties. Two characteristics of LDH virus infection are sufficiently unique to be considered definitive for identification: 1) Five- to 10-fold elevation of plasma LDH level occurring within 2 to 4 days after inoculation and persisting indefinitely, and 2) development of high titer viremia (10^9 to 10^{11} ID_{50}/ml) at 24 hours, with persistence of viremia at a lower level (10^4 to 10^5 ID_{50}/ml) throughout life(3,4).

A subline of the WM1 strain, "pure WM1," which had been freed of contamination with "avirulent WM1-B virus"(2,5) was studied in these respects. In addition, studies were made of the occurrence of similar agents in house mice in Maryland.

Materials and methods. Mice of the Q.I.M.R. Stock infected with 10th passage of "pure WM1" virus were sent to Bethesda from Brisbane, and subsequent passages and experiments were done in NIH Swiss mice, a strain shown to be free of LDH virus. Inocula were prepared by homogenizing liver and spleen in basal Eagle's medium containing 20% veal infusion broth (BME-VIB) and centrifuging at 2000 rpm/15 minutes. The supernate was again centrifuged at 8000 rpm/5 minutes. On occasion plasma was used for passage. Viral assay based on LDH rise was done by inoculating weanling mice intraperitoneally (i.p.) with 0.1 ml of test material. Each test included 3 to 5 uninoculated mice; occasionally, mice inoculated with normal NIH mouse tissue were included as additional controls. Four to 6 days later, blood was individually collected from the orbital plexus by heparinized capillary pipettes (A. H. Thomas, Philadelphia, Pa.), centrifuged at 2000 rpm/20 min, and the plasma removed. When animals were to be held for later LDH or virus assay, the experimenter cleaned the work area and washed his hands between groups of animals. If tests for the estimation of plasma LDH were not conducted on the day of collection, the plasma samples were stored at —20°C until tested. Plasma LDH levels were determined by a colorimetric method(6) modified for use in the Microtiter® system, (Cooke Engineering Co., Alexandria, Va.) using disposable plastic plates and droppers and loops delivering 0.025 ml. Two-fold dilutions of plasma from 1:2 to 1:256 were made in 0.1 M phosphate-buffered saline pH 7.4. Three drops of a substrate and coenzyme solution (8 mg sodium pyruvate and 100 mg reduced diphosphopyridine nucleotide in 100 ml of pH 7.4 buffer) were added to each well and the plate was held at 37°C for 1 hour. One drop of color reagent (20 mg 2,4 dinitrophenylhydrazine, 7.5 ml conc. HCl and distilled water to final volume of 100 ml) was added, the plates were shaken, and after 15 minutes at room temperature one drop of 5N NaOH was added. Clear tape was placed over the top of the plate to seal each well individually, and the plates were again shaken. After a few minutes the reactions were read visually and the degree of decolorization rated on a 0 to ++++ scale. A negative control serum was included in each test. A comparison of titers obtained by the plate colorimetric method and the reaction rate measured by the standard spectrophotometric method indicated close correlation. Although not suitable for highly quantitative studies of lactic dehydrogenase levels, the plate method proved quite reliable as a rapid diagnostic test.

Experiments on recovery of virus from wild mice were carried out in an area separate from other experimental animals and procedures.

Results. Preliminary studies indicated that infection of NIH mice with undiluted "pure WM1" preparation resulted in mild splenomegaly in a small proportion of mice. Five of 20 inoculated mice examined at one month had spleens weighing 200 mg or more, while none of 20 mice inoculated with normal NIH mouse tissue extract had spleens as large as 200 mg. However, all infected mice demonstrated increased plasma LDH levels within 3 days after inoculation, the elevation persisting for at least 27 days. The levels of LDH activity were comparable to those observed by the same assay procedure in prior

TABLE I. Association of LDH Activity and Production of Splenomegaly in Passage Lines Established from Limiting Dilutions of a Titration.

Passage		Dilution of "pure WM1" pool 10^{-7}	10^{-8}	10^{-9}	10^{-10}	Normal NIH mouse liver-spleen
1	LDH increase	5/5*	3/4	1/5	0/4	0/5
	Splenomegaly	NR†	NR	NR	NR	0/36‡
2§	LDH increase	NT	5/5	5/5	0/5	NT
	Splenomegaly	NT	4/19	2/20	2/20	NT
3	LDH increase		5/5	4/5	0/4	
	Splenomegaly		8/39	7/40	0/40	

* Proportion of mice with elevated plasma LDH level.
† NR = not recorded. NT = not tested.
‡ Proportion of mice with spleen weight $\geq$ 200 mg.
§ Passages 2 and 3 were made with 10% organ suspensions.

studies of a strain of LDH virus obtained from Dr. Riley. A suspension of liver and spleen harvested 32 days after inoculation induced LDH rise when diluted 10^{-5}, and tissues taken 5 months after inoculation also induced LDH rises in NIH mice.

To determine if WM1 virus induced the early viremia characteristic of LDH virus, 3 serial passages were made at 24 hour intervals by inoculating plasma intraperitoneally. The pooled plasma of the final group, harvested at 24 hours, contained an agent which stimulated LDH rise, in a titer of $10^{9.5}$ ID_{50}/ml.

It was thus established that the "pure WM1" virus possessed an activity which was biologically indistinguishable from LDH virus. Two explanations could be considered. It was possible that the WM1 virus, defined as producing moderate splenomegaly in mice, was also responsible for the elevation of plasma LDH. Alternatively, 2 viruses might be present, one (WM1) producing splenomegaly and the other (LDH) producing the enzyme effects. An attempt was made to resolve this problem by determining whether high dilutions of the "pure WM1" pool possessed the capacity to produce splenogegaly as well as to increase plasma LDH levels.

The dilutions about the endpoint of a virus titration were tested for capacity to produce splenomegaly in mice. Two serial passages at monthly intervals were made from the 10^{-8}, 10^{-9}, and 10^{-10} dilutions (Table I). LDH virus was present in the passage material from the 10^{-8} and 10^{-9} dilution. The spleen sizes of the mice receiving the dilutions were not compared as the groups were small; no significant differences were detected in the second passage. However, in the third passage there was a higher incidence of moderate splenomegaly in the 2 groups of LDH-infected mice than in either of the 2 negative groups (one being the third passage from the 10^{-10} dilution and the other a group of mice inoculated with liver and spleen of normal NIH mice). This splenomegaly was similar in degree and incidence to that observed in NIH mice infected with low dilutions of "pure WM1" virus. Although these results provided information of an indirect nature, it suggested that this strain of LDH virus was capable of causing moderate splenomegaly and that it was the component of the "pure WM1" line responsible for the production of both splenomegaly and enzyme increases. In other words, it appeared that the "pure WM1" virus was actually LDH virus.

Isolation of LDH virus from wild mice. As the WM1 virus had been isolated from a wild mouse, an attempt was made to isolate LDH virus from wild mice in this country. The tissues of a series of wild mice (*Mus musculus*) captured on a chicken farm in Montgomery County, Maryland, had been stored whole at —60°C for 2 years. A pool of spleen plus thymus of each individual mouse was made into an approximately 10% suspension in BME-VIB and centrifuged at 2000 rpm for 15 minutes. Each suspension was inoculated i.p. (0.1 ml) into 2 weanling NIH mice and intracerebrally (0.03 ml) into another 2 for detection of LCM virus.

Plasma samples for LDH determination were taken from all mice at 7 days and again at 24 days. Suspensions from 8 individual wild mice were tested, and 4 were found to induce persistent elevation of plasma LDH levels in all recipients. The presence of lymphocytic choriomeningitis (LCM) virus in the wild mouse tissue suspensions was excluded by demonstrating that mice inoculated intracerebrally showed no signs of illness and that all mice (i.p. and i.c.) were completely susceptible to subsequent challenge with LCM virus.

The 4 positive suspensions were retested by inoculation into 2 fresh NIH mice; pooled plasma of each recipient group was taken at 24 hours and titrated for LDH virus. In all 4 groups the plasma titered $10^{8.5}$ to 10^{9} ID_{50}/ml. Thus, these tests established that the wild mice contained transmissible agents producing 3 definitive properties of LDH virus infection: elevation of LDH level, persistence of the elevated LDH level, and early high titer viremia.

Discussion. Since serologic identity is the definitive test for identification of viruses, it is difficult to establish identity between agents for which no serologic test is available, and whose signs of infection are not highly specific. However, the highly unique features of infection with LDH and WM1 viruses, as well as the general properties of the viruses are so similar that their identity must be considered as being as clearly established as that of the various strains of LDH virus recovered from mouse tumors.

It is interesting that the LDH virus was discovered independently in 3 laboratories using entirely different endpoints, *i.e.*, LDH enzyme rise(3), splenomegaly(1), and interference with vesicular stomatitis virus infection in infant mice(7).

Previous studies of the mouse passage line of WM1 virus resulted in detection of a number of viral agents, some of which may have been acquired during passage. These include WM1-B, which produces a disease similar to Friend virus infection(5); a nonleukemogenic virus related to WM1-B as indicated by neutralization tests(2); and possibly a lymphatic leukemia virus(1). The "pure WM1" virus studied here had been freed of avirulent WM1-B by passage with antiserum.

As splenomegaly was regularly observed throughout serial passages of the "original WM1" virus, it is assumed that the virus present in later passages (and considered to be LDH virus) was the same as that isolated from the wild mouse. Further evidence that such a virus producing splenic hyperplasia was indeed present in wild mice in Queensland existed in the isolation of several strains from wild mice(2), with splenic hyperplasia sometimes evident in mice of the first few passages. No such virus has been isolated from any other source during the course of many blind passages in mice of the same stock. It was therefore highly suspected that wild mice carried LDH virus, and this was readily confirmed by the isolation of the virus from wild mice in Maryland.

The great majority of isolates of LDH virus have been obtained as contaminants of laboratory-passaged tumors or virus stocks. Spontaneous infection of laboratory mouse colonies appears to be rare, although the virus was widely disseminated in the one colony known to be infected(8). The finding of LDH virus in wild mice indicates that they may provide an important reservoir of the virus, and may be responsible for its occasional introduction into laboratory materials.

Summary. WM1 virus, a spleen-enlarging virus recovered from wild house mice in Australia, is a strain of LDH virus. Four of 8 wild mice trapped in Maryland yielded LDH virus.

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Effect of Endotoxin on Macrophages of the Adult Chicken. (29437)

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Endotoxin has been shown to have a marked toxic action *in vitro* on splenic macrophages of the guinea pig, rabbit, and man (1). Tissue culture methods have also been used to demonstrate a severe toxic effect on macrophages of the spleen and lung of the rabbit during an 8-hour period following an intravenous injection of endotoxin(2). The significance of these observations has been discussed elsewhere(3).

Avian species appear to be more resistant to endotoxin than mammalian species. Ball *et al*(4) found turkey poults resistant to intravenous injection of 1 to 15 mg of endotoxin. They also observed that hens tolerated the administration of 500 μg of endotoxin without the occurrence of hemorrhage into tumors associated with fowl leukosis. Recently Jordan and Hinshaw reported that adult chickens survived intravenous inoculation of 10 mg/kg of endotoxin(5). Several *in vitro* studies with peripheral blood leucocytes of the chicken indicated that endotoxin had a positive chemotactic effect(6-8) and stimulated leucocyte migration(6-11). Growth in cultures of chick embryo tissues has been found to be unaffected by high concentrations of endotoxin(12-14).

The purpose of the present study was to observe the relative sensitivity of macrophages from adult chickens to different concentrations of endotoxin *in vitro*. The use of culture techniques employed in similar studies on mammalian tissues permitted a comparison of the response of mammalian and avian macrophages.

Material and methods. White Leghorn cockerels from a pullorum-clean flock* maintained on an antibiotic-free diet provided the source of tissue and of blood used in preparation of media. The lot of endotoxin employed was the same as that used in a previous study on mammalian tissues(1). This was a sample of endotoxin from *Escherichia coli* 0127:B8 (Boivin type, Difco). A 0.1% suspension in Tyrode's solution was heated in a water bath at 70°C for one hour. Further dilutions were made in Tyrode's solution for each experiment. Tissue culture methods employed were similar to those previously described(1). Blood was obtained from etherized cockerels by cardiac puncture and 0.5 unit/ml of heparin was added to a portion of the blood for the preparation of plasma. Tissues were removed under sterile conditions and explants prepared and matched for size. Twelve fragments were used for each experimental condition. Streptomycin (100 units/ml) and test substances were added to the medium as in previous studies(1). Cultures, made in D5 Carrel flasks, consisted of 0.5 ml of plasma, 1.0 ml of serum extract of chick embryos and 4 explants placed in the medium before coagulation occurred. The extent of migration of small and large wandering cells was measured with an ocular micrometer at intervals during incubation in a manner previously described(1). Coverslip cultures were prepared for histologic examination and were fixed in Zenker-formol solution at 24 hours of incubation and stained by a modification of the Dominici technique(15).

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