factor is not present in the patient's blood, her genotype may be represented as Tj^bTj^b and paradoxically she has been harboring, at least in her tumor cells, the Jay gene Tj^a not transmitted from either of her two parents. In the absence of external sources for the blood factor in the tumor cells, one is led to assume that its unexpected presence may be brought about by intrinsic changes in the body, *i.e.*, a genetic mutation.

If only one locus—the site of the gene for the blood factor—is affected, then the mutation may be held responsible for the presence of the blood factor in the tumor. Because mutations have been frequently discussed in the pathogenesis of malignancy, one is tempted to suggest that the mutation may have also induced the tumor. This question, however, must be left open, in view of the importance of the issue involved and the absence of any direct evidence.

In any event, the highly exceptional circumstances in the case here reported provided specific agglutination reactions, the interpretation of which has led to a rather unexpected conclusion that a blood factor in tumor cells may arise from a genetic mutation.

‡ For production of immune isoagglutinins resulting from transplantable tumors in mice and rats cf. Gorer(2) and Lumsden(3). Isoimmunization by tumor cells is also a possible explanation for the origin of a very weak anti-Duffy which caused a hemolytic transfusion reaction at the first transfusion of a 64-year-old patient suffering from benign prostatic tumor. This case was recently reported by Rosenfield, Vogel, and Race(4) who made no comments on the origin of a presumably immune antibody in the pre-transfusion specimen.

2. Gorer, A. P., *J. Path. and Bact.*, 1937, v44, 691.

3. Lumsden, T., *Am. J. Cancer*, 1938, v32, 395.

4. Rosenfield, R., Vogel, P., and Race, R. R., *Rev. D'Hem.*, 1950, v5, 315.

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Excretion of Coxsackie Virus (Conn. No. 5 Strain) in the Urine of Infected Mice.* (18795)

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In the already abundant literature on infection with viruses of the Coxsackie group, we have found but one casual reference to the recovery of virus from the urine of human cases(1). Howitt, in listing the sources from which Coxsackie virus had been recovered includes urine from one case. No details are given. There have been no reports concerning the elimination of virus in the urine of infected animals. Because of the possible epidemiologic importance, the following observations demonstrating viral excretion in the urine of infected mice seem worth placing on record.

Exp. I. Several 0-day-old mice were inoculated intraperitoneally with 10^{-3} dilution of Conn. No. 5 passage virus in buffered saline. After 48 hours, at which time the mice showed the usual signs of illness, urine was obtained from several animals by direct aspiration of the bladder with a tuberculin syringe. The urine was kept frozen in the syringe for 5 days, then thawed, and penicillin and streptomycin (500 units per cc) added. The final dilution was approximately 1 to 3. Ten mice, 1 to 2 days old, were inoculated intraperitoneally with 0.02 cc of this diluted urine. Three days after injection, 9 of the 10 mice were dead or missing; the moribund survivor was sacrificed, and a 10% suspension of carcass, to which 500 units of penicillin and streptomycin per cc had been added, was frozen for further passage. Liver and pancreas were examined histologically and showed the usual lesions found in suckling mice infected with the Conn. No. 5 strain

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1. Howitt, B. F., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 443.

of Coxsackie virus(2). The 10% suspension of carcass of the suckling mouse, from which skin, feet, tail and gastrointestinal tract had been removed, was diluted in 15/100 *M* phosphate-NaCl buffer to 10^{-3}, and inoculated into nine 0-day-old mice. All were dead on the 4th day.

Exp. II. Again, Conn. No. 5 passage material, in dilution of 10^{-2}, was inoculated intraperitoneally into 3 mixed litters of 0-day-old mice. After 48 hours, urine was collected by aspiration of bladder with a syringe. The urine, to which penicillin and streptomycin had been added, was diluted 1 to 10 with phosphate-saline buffer solution and frozen. After 4 days, the urine was thawed, and 0.02 cc of 10-fold dilutions injected intraperitoneally into 0-1-day-old mice. All mice given the highest dilution—10^{-5}—succumbed to the infection. Histologic examination of mouse No. 4394, killed when moribund on the 6th day after injection of 10^{-3} dilution showed interstitial pneumonia and early necrosis and calcification of cervical fat.

A second titration of urine was made to determine the end-point, with the results shown in Table I.

The $I.D._{50}$ as calculated by the Reed-Muench method, was $10^{-5.6}$. The virus is thus shown to be present in high concentration in the urine.

Controls. Eight 0-day-old mice were inoculated intraperitoneally with 0.2 cc of urine obtained from normal 2-day-old mice, killed by decapitation. One mouse was found dead on the following day, but showed no microscopic lesions. Another was missing on the 3rd day. A healthy survivor, sacrificed on the 6th day, had normal organs. The 5 surviving mice remained well.

Presence of virus in the urine of adult mice infected with Conn. No. 5 virus. Although adult mice have been regarded as not being susceptible to infection with the Coxsackie group of viruses, it has been found in this laboratory that the Conn. No. 5 strain regularly produces severe lesions in the pancreas, and that the virus can readily be maintained

TABLE I. Infectivity of Urine for Suckling Mice.

Dilution	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Results	8/8*	8/8	6/9	2/9	1/9	1/9	1/9

* Numerator—No. of dead mice; denominator—total No. of mice inoculated.

in adults by serial passage of pancreas suspensions. A full account of these observations will be published elsewhere(3). It was, therefore, thought of interest to ascertain whether this virus might also appear in the urine of infected adults. Accordingly urine was collected on the 4th day after intraperitoneal inoculation of female mice averaging 15.6 g in weight at the beginning of the experiment. All the animals had lost weight, and were proven to have pancreatic disease when killed. This was the 4th passage of the virus in adult mice. Nine 1-day-old mice were inoculated intraperitoneally with 0.02 cc of urine. Five mice survived for 22 days, without showing signs of illness. One mouse, found dead on the 5th day, was not examined. Two were sacrificed for histologic study. One of these was killed on the 5th day. No characteristic lesions were found. The second mouse, however, when sacrificed on the 11th day, had marked tremors and was obviously sick. There was found diffuse encephalitis and myelitis; the lesions were identical with those in the central nervous system of Conn. No. 5 infected sucklings surviving for a corresponding period. Also there was extensive necrosis with calcification in the cervical and interscapular fat pads.

A second experiment confirmed the fact that virus may at times be excreted in the urine of infected adult mice. Pooled urine from 11 young adult mice was collected on the 4th, 6th and 7th day. They had been inoculated intraperitoneally with 0.1 cc of 10^{-1} suspension of carcass of infected sucklings, and had shown when killed the usual weight loss and gross pancreatic lesions. The 4-day specimen was contaminated, and the mice injected with it died of bacterial infection. The urine obtained on the 6th day failed to produce characteristic signs and lesions, when

2 Pappenheimer, A. M., Daniels, J., Cheever, F. S., and Weller, T. H., *J. Exp. Med.*, 1950, v92, 169.

3. Pappenheimer, A. M., Kunz, L. J., and Richardson, S., *J. Exp. Med.*, in press.

inoculated into new-born mice. The specimen secured on the 7th day, however, was found to contain virus, all 10 of a litter dying or being sacrificed when showing obvious illness. Three of them were proven, on histologic examination, to have typical lesions of myocardium, liver and pancreas. A fourth was frozen, and after 10 days storage in the deep freeze, proved infective when injected intraperitoneally in 10^{-1} dilution into 0-day sucklings. All the animals died on the 3rd day. Three examined histologically proved to have characteristic pancreatitis and hepatitis.

This experiment proves that Connecticut No. 5 virus may be excreted in the urine of infected adult mice. The optimal period and concentration remain to be determined.

Exp. III. That the lesions produced by inoculation of urine from infected mice are due to the Conn. No. 5 virus and not to non-specific toxic substances is indicated by the fact that the addition of immune anti-Conn. No. 5 serum to the infected urine for one hour before inoculation confers at least partial protection.

Infected urine + Conn. #5 antiserum	.02 cc	3/8
" " + normal mouse serum	"	8/8
" " + anti-Ohio R serum	"	8/8

The 3 deaths in the first group occurred within the first 2 days after injection and were probably non-specific. Similar early deaths have occurred in mice inoculated with normal urine.

Conclusion. Infant mice infected with the Conn. No. 5 strain of Coxsackie virus eliminate virus in the urine. The infectious titer of urine obtained on the 2nd day after intraperitoneal inoculation is $10^{-5.6}$. Adult mice with pancreatic disease caused by Conn. No. 5 virus may also excrete virus in the urine.

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Production of Cytoplasmic Azurophilic Inclusions in Connective Tissue Cells by Suspending Agents.* (18796)

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During recent studies on the action of cortisone acetate (Cortone-Merck) on acute localized antigen produced inflammation in the mouse(1), it was observed that cytoplasm of fibroblasts of loose connective tissue of treated mice contained acidophilic inclusions (2,3). The purpose of this investigation was to obtain information on the origin and nature of the inclusions and on the mechanism underlying their formation.

TABLE I. Changes in Percentage Distribution of Fibroblastic Inclusions in Cells of Loose Connective Tissue of Intact Mice Produced by Cortone (Merck).

	No. of animals	Mean % of fibroblasts containing cytoplasmic inclusions and S. E. 4 hr	24 hr	48 hr	96 hr	6 days
1 mg Cortone s.c. in 2 cc suspending agent	20	28 ± 9.87	40.6 ± 6.28	36.6 ± 5.58	34.6 ± 4.59	26.5 ± 5.63
2 mg Cortone s.c. in 4 cc suspending agent	20	36.5 ± 7.15	52.5 ± 4.47	48 ± 4.87	42.6 ± 5.68	28.2 ± 4.65
1 mg crystalline cortisone acetate s.c. in .2 cc saline	20	0	0	0	0	0

* Aided by grants from the Life Insurance Research Foundation and the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

1. Dougherty, T. F., and Schneebeli, G. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 854.

2. Schneebeli, G. L. (Abst.). *Anat. Rec.*, 1950, v106, 78.

3. Schneebeli, G. L., Loewe, S., and Dougherty, T. F. (Abst.) *Anat. Rec.*, 1951, v109, 122.