TABLE II. Lipid Composition of Epidermis and Dermis as Influenced by Hair Growth Cycle.

Tissue		Cholesterol esters, %	Cholesterol, %	Triglycerides	Iodine value, Wijs
Dermis-anagen*	(9)	.27 ± .06	.75 ± .2	99 ±.16	71 ±13
Epidermis-anagen*	(5)	2.3 ± .6 †	3.1 ± .5	95 ±.6	59 ± 3
Dermis-telogen*	(6)	.44 ± .09	.5 ± .14	99.1±.2	63 ± 8
Epidermis-telogen*	(4)	1.8 ± .4 ‡	2.1 ± .4	96 ±.4	62 ± 4

* Anagen and telogen dermis and epidermis as indicated in Table I. No. of samples analyzed indicated in parentheses.

† Fast acting sterols were 18% of esters, 9.4% of total steroids.

‡ " " " " 27% " " , 13% " " " .

of the hair growth cycle represent times of maximum morphological differences in thickness of the corium and adipose layers. Yet total lipid, phospholipid, neutral lipid, cholesterol esters, cholesterol and triglyceride content of epidermis during anagen VI and telogen stages of the hair growth cycle are not significantly different. The same is true for the lipid composition of the dermis. However, independent of hair growth activity, epidermis has a lower water content and a much greater level of cholesterol, cholesterol esters and phospholipid than dermis. The fast acting sterols of epidermis are limited largely to the esterified fraction.

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A Heat-Stable Hemagglutinin in Mouse Tissue Extracts.* (26076)

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It has been observed repeatedly that hemagglutination by the polyoma virus takes place at "refrigerator," but not "room," temperature(1). In our laboratory we have been investigating the possible presence of this agent in normal and neoplastic mouse tissues, using hemagglutination *in vitro* as a method of identification(2). This paper describes a heat-stable hemagglutinin, derived from extracts of mouse tissues and active at both 4° and 23°C, which was demonstrated in the course of these experiments.

Materials and methods. Four kidneys, 26 livers, 2 lungs and 6 mammary glands were obtained from 34 mice of 20 inbred strains.

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TABLE I. Agglutination of Mouse Red Blood Cells by Tissue Extracts (Fraction 1, Fraction 2 and Fraction 3).*

Tissue extracted	Extracts*	Titers† With nonheated extracts 4°C	With nonheated extracts 23°C	With heated extracts 4°C	With heated extracts 23°C
Normal mouse	Fraction 1	0	0	80–320	80–640+
	" 2	0–40	0–40	0–80	0–320
	" 3	10–40	10–320	10–160	40–640+
Mouse tumors	" 1	0–20	0	0–320	0–640+
	" 2	0	0	0–80	0–320
	" 3	20–160	20–160	40–640+	160–640+
Beef liver, kidney; lamb liver	" 1	0–640+	0–320	80–160	20–160
	" 2	0	0	0–160	0–160
	" 3	640+	20–80	80–160	40

* Fraction 1, supernatant after 550 × g; Fraction 2, supernatant after centrifugation of Fraction 1 at 105,000 × g; Fraction 3, pellet after centrifugation of Fraction 1 at 105,000 × g.
† Titers shown as reciprocals of extract dilutions. 0 = no agglutination. Red cell-extract mixtures incubated at temperatures shown.

Thirteen autochthonous mammary adenocarcinomas, 5 autochthonous leukemias, 2 transplanted leukemias, a transplanted melanoma and a transplanted squamous cell carcinoma were obtained from a hybrid mouse and 21 mice of 7 inbred strains. Tissue extracts were prepared as follows: a 10% suspension of tissue in Hank's solution was homogenized with a glass homogenizer and motor-driven Teflon pestle. The homogenate was spun at 550 × g for 10 minutes in an International refrigerated centrifuge (Model PR-1). An aliquot of the supernatant was removed (Fraction 1) and the remainder spun at 105,000 × g for 35 minutes in a Spinco Model L centrifuge. The supernatant (Fraction 2) was saved and the pellet (Fraction 3) resuspended (1.0 ml/g of original tissue) in phosphate buffered saline (pH 7.2). Materials were handled aseptically and kept chilled at all times. All tissue extracts were stored for varying intervals at −60° to −70°C prior to testing. Whole blood, obtained from young C57BL/Ks mice by tail bleeding and from adult guinea pigs by heart puncture, was collected in Alsever's solution. Red blood cell suspensions were prepared by the method described by Hartley *et al.*(3). Hemagglutination tests were run in Kahn tubes using serial 2-fold dilutions of tissue extracts in buffered saline (pH 7.2). Each tube, containing 0.2 ml of the diluted extract, received 0.2 ml of a 1.0% suspension of washed red cells. Both nonheated and heated preparations (56°C for 30 minutes) were used. The tests were carried out at room temperature (23°C) and in the cold (4°C); readings were made after 2 to 12 hours by the pattern method.

Results. Agglutination of mouse erythrocytes by normal mouse organs. Table I summarizes data obtained when extracts of normal mouse organs were tested for agglutinating action with mouse red cells. The agglutinating effect could be altered by several factors. Nonheated preparations yielded similar titers when incubated at 4° or 23°C, but were less active than heated preparations (56°C for 30 minutes) at either incubation temperature. The highest titers were obtained with heated extracts incubated at 23° C. The importance of centrifugation is shown by a comparison of agglutination titers obtained with the 3 tissue extracts. In general, Fraction 1 and Fraction 3 gave higher titers than Fraction 2. These results suggest that the heat-stable agglutinin does not sediment with low-speed centrifugation (500 × g for 10 minutes), but is partially sedimented by centrifugation for 35 minutes at 105,000 g. Some variability in titer was noted when comparisons were made among extracts of the different organs. Highest titers were obtained with liver and lowest with mammary gland. Comparable titers were observed with extracts of lung and kidney; these values occupied a median position.

Agglutination of mouse erythrocytes by

mouse tumors. Titers of extracts of mouse neoplasms incubated with mouse red cells were comparable to those observed with normal mouse organs (Table I). Further, similar effects of centrifugation, incubation temperature, and heating on titer were noted. Titers with extracts of transplanted tumors (leukemias, squamous cell carcinoma and melanoma) were low. Those observed with autochthonous leukemias and mammary adenocarcinomas were similar to the titers found with normal liver extracts.

Agglutination of mouse erythrocytes by extracts of beef and lamb tissues. Consideration was given to the possibility that the heat-stable agglutinin might be present in tissues of species other than the mouse. Beef liver and kidney, and lamb liver were supplied by a local market; tissue extracts were prepared as above. Titers were obtained with these preparations, but they were quite variable (Table I). Centrifugation and temperature of incubation did not alter the agglutinating effect in a predictable fashion. In contrast to results obtained with mouse tissues, heating at 56°C for 30 minutes effected a reduction, rather than an increase, in titers. Considerable variability in titers was also evident when comparisons were made among the different tissue fractions.

Agglutination of guinea pig erythrocytes. Tests for agglutination of guinea pig red cells by tissue extracts (normal mouse organs, mouse tumors, beef and lamb tissues) were carried out on a representative number of extracts. Routinely, the titers were low when compared with those obtained with mouse erythrocytes. Extracts of mouse tumors had little or no agglutinating effect, but in general, the results reported above with mouse erythrocytes are applicable here.

Effect of filtration on agglutination. Low-speed supernatants (Fraction 1) of mouse tissues, made as described previously, were prepared from normal C57BL/Ks livers, autochthonous mammary adenocarcinomas from A/Sn and ST/Ks donors, and AKR/J donors with autochthonous leukemias (liver, spleen, thymus, lymph nodes). Aliquots were saved and remaining supernatant materials filtered (Selas porcelain candle, No. 03). The agglutinating action of these extracts on mouse erythrocytes is summarized in Table II. The effect of heating on agglutination titers is evident. Heated filtered extracts had a lower agglutinating effect than heated nonfiltered preparations (Fraction 1) at either incubation temperature.

TABLE II. Agglutination of Mouse Red Blood Cells by Tissue Extracts (Fraction 1 and Filtrate).*

Tissue extracted	Extracts*	Titers† With nonheated extracts 4°C	23°C	With heated extracts 4°C	23°C
Normal mouse liver	Frac. 1	0	0	320	320
	Filtrate	0	0	80	160
Autochthonous *mammary ca.*	Frac. 1	0	0	320	320
	Filtrate	0	0	20	20
Autochthonous leukemia	Frac. 1	80	40	320	160
	Filtrate	0	0	80	40

* Fraction 1, supernatant after 550 × g; Filtrate, prepared by filtration of Fraction 1 (03 Selas porcelain candle).

† Titers shown as reciprocals of extract dilutions. 0 = no agglutination. Red cell-extract mixtures incubated at temperatures shown.

Failure to reverse agglutination. Mouse tissue extracts and mouse erythrocytes were incubated at 4°C for 2 hours. Titers were recorded, and the cells were then washed 3 times in chilled buffered saline (pH 7.2). Supernatants of the third washing were drawn off and mixed with fresh red cells; the remaining sedimented cells were resuspended in fresh saline. Both sets of tubes were incubated at 37°C for 30 minutes, then at 4°C for 2 hours. There was no reversal of agglutination; approximately 15% of the heat-stable agglutinin was eluted.

Variable ranges in agglutination titers. Consistent titers with a given sample were observed in all but a few instances. Negative results were obtained from experiments designed to test the possibility that repeated freezing and thawing of the tissue extracts or aging of the red blood cells might account for the occasional variability.

Discussion. Salaman(4) described hemagglutination of mouse erythrocytes by extracts of normal and cancerous mouse tissues. Later, Gross(5) reported that mouse red blood cells are agglutinated by passage A leukemic fil-

trates. The present study extends these experiments by demonstrating the presence of a heat-stable agglutinin in extracts of normal, as well as neoplastic mouse tissues. The identity of the heat-stable agglutinin has not been ascertained, nor has it been possible to determine whether the activity of these preparations is due to more than one agglutinating agent. However, the data support the concept of a hemagglutinin which is not a tumor virus. The heat-stable agglutinin differs from the polyoma virus (*cf.*(6)) in 3 ways: (a) only a slight elution (approximately 15%) of the heat-stable agglutinin occurs at 37°C; (b) mouse red cells give higher agglutination titers than guinea pig erythrocytes; and (c) agglutination takes place both in the cold (4°C) and at room temperature (23°C).

Fundamental differences exist between the heat-stable agglutinin and the hemagglutinating agents reported by Salaman(4). Significant differences are not apparent, however, when a comparison is made between the agglutinating action of passage A leukemic filtrates(5) and our mouse tissue extracts, whether of normal or cancerous tissues. Gross(5) poses the question as to "whether the agglutinating potency of leukemic extracts is actually related to presence of the leukemic agent." Comparison of our results obtained with filtered and nonfiltered extracts of mouse tissues shows that filtration effects a marked reduction in agglutination titers. These findings are contrary to what one would expect if a viral agent were involved.

The agglutinating activity which we observed with extracts of beef and lamb tissues was not anticipated. The data indicate that agglutinin(s) in the beef and lamb preparations is neither a serum antibody, since high titers were obtained with Fraction 3, nor analagous to the heat-stable agglutinin derived from mouse tissues. In addition, these observations point out the need for caution before stating that the agglutinating action of a given tissue extract is due to a tumor virus.

Summary. Extracts of normal and neoplastic mouse tissues were found to agglutinate mouse and guinea pig red blood cells. Characteristics of the heat-stable substance(s) responsible for this phenomenon are discussed and it is proposed that the hemagglutinating agent(s) is not a tumor virus.

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Urobilinogen Excretion After Hemoglobin Infusions in Patients with Normal Hematologic and Hepatic Findings.* (26077)

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According to Watson(1), the normal daily excretion of urobilinogen in feces ranges from 40 to 280 mg, averaging 140 mg in a recent study. In the urine, daily normal excretion is from 0.5 to 1.5 mg. Theoretically, it is possible to calculate rate of hemoglobin destruction from daily excretion of urobilinogen on the assumption that 1 mg of urobilinogen is derived from 28.5 mg of destroyed he-

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