a globule overlay an arterialized sinusoid and pulsated back and forth with the onrushing and receding pulse wave beneath it. It was obvious at this magnification that the fat droplets definitely pressed on the sinusoids and impeded the passage of blood. When after hours of observation the continuous flow had slowed and become granular in character one could see red cells squeezing between opposing fat globules.

In 3 other rats studied after 48-96 hours of choline deficiency the changes were more accentuated. One had the impression that the tissue had been strewn with round transparent pellets that covered and distorted the sinusoids making the flow harder to follow. The central veins although slender still showed good flow coming from the deeper layers.

A group of 6 rats on a choline deficient diet for 5 to 7 days were studied by the same technic. Under low magnification (X 50) the sinusoidal pattern had almost faded. It could be made to reappear by raising the insufflation pressure of the O_2 to about 60 mm Hg. This pressure impeded the outflow of blood from the hepatic veins and the blood stagnated, dilating the hepatic sinusoids. Under medium magnification (X 100) the hepatic cells appeared fatty and some looked as if they were translucent bubbles. Rows of small globules were tightly packed against the sinusoidal walls. The red streaks representing the flowing blood in the sinusoids were narrow and had a conspicuously indented outline due to the compression of the sinusoidal walls by the fat globules. The smaller the globules the less compressible they were.

Summary. (1) A method has been developed by which intraabdominal pressure sufficient to immobilize the liver can be established in small animals laparotomized for the study of the intrahepatic circulation *in vivo*. (2) In choline-deficient rats there is an accumulation of tiny fat droplets along the sinusoidal walls or overlying them as early as 24 or 36 hours after the ingestion of the deficient diet. The small and barely compressible droplets exert a pressure on the pliable endothelial walls indenting them and impeding the sinusoidal flow. (3) These phenomena are conspicuous on the surface of the transilluminated liver edge which of course is formed by zones 3 of the acini under observation.

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The Penn Seroflocculation Reaction in Cancer Diagnosis.* (23794)

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A seroflocculation reaction was reported by Hall, Dowdy, Penn, and Bellamy(1) to have significant cancer selectivity, *i.e.*, in testing over 10,000 sera their correct positive results in the cancer patients ranged between 81 and 98%. Their false positive results in testing patients ranged between 9 and 33% while their false positive results in testing normal persons ranged between 0 and 4%. These results were obtained using 3 different "anti-

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gens" (A, B, & C) over a period of 10 years. Using a still different "antigen" (D), presumably ethyl choladienate, they found 100% correct positive results in testing 80 cancer sera, 57% false positive results in testing 85 non-cancer sera, and 3.8% false positive results in testing sera from 103 normal individuals. At the invitation of Dr. Dowdy, several laboratories in different parts of the country undertook a group study of the Penn seroflocculation reaction using ethyl choladienate as antigen. Our participation consisted of a clinical trial of the reaction using sera from patients in the University of Kansas Medical Center. This report describes the results obtained in testing 241 cancerous and 1,608 noncancerous patients as well as 129 normal ambulatory individuals. Although the positive results in the normal group were only 5% and the 12% positive results in the non-cancer group were not excessive, the procedure was not a satisfactory cancer test since it was positive in only 43% of the cancer group.

Method. The seroflocculation procedure is based on the reaction between serum and an "antigen" composed of buffered saline, cholesterol and ethyl choladienate. The qualitative test which is routinely performed uses 2 different ratios of serum to antigen. A positive reaction in either tube is considered indicative of cancer and is recorded when aggregates form and the liquid phase becomes less turbid. Varying degrees of turbidity without particle formation are recorded as negative results. Instructions and practice in performing the test for staff from this laboratory were kindly provided in the laboratories of Hall and Penn. The method was that published for "antigen" D by Penn and Hokama(2). The antigen was ethyl choladienate supplied to us by Hall. The seroflocculation test light recommended by Hall was used in estimating the degree of aggregation in the tubes. All sera were obtained from patients in the University of Kansas Medical Center and were tested without knowledge of the diagnosis. Classification of the patients was carried out several months later after careful review of the completed clinical record. The test results and the clinical classification data were forwarded to Dr. J. E. Dunn, Jr. of the U.S. Public Health Service in Berkeley, Calif., for analysis and correlation with similar data produced by the other laboratories. The incidence of the various types of nonmalignant disease is shown in Table I. The numbers of patients tested in each category are shown in Part A of Table I, while Part B gives the numbers after deletion of certain classes of patients known to have a high incidence of false positive results, *i.e.*, those having hay fever or asthma, rheumatism or arthritis, active tu-

TABLE I. Incidence of Positive Results in the Various Types of Non-Malignant Disease and in the Normal Group.

	A. Without deletion			B. With deletion of certain patients*		
Type of disease	No. of patients	No. & % patients with positive results		No. of patients	No. & % patients with positive results	
Minimal disease	313	17	5	312	17	5
Types of inflammation	303	98	32	189	33	12
Heart disease	243	51	21	211	32	15
Types of hyperplasia	121	15	12	117	14	12
Ophthalmic cases	119	16	13	109	12	11
Benign tumor	84	9	11	80	6	8
Diabetes mellitus	74	19	26	70	17	24
Pregnancy	71	28	39	0	0	
Gastric & duodenal ulcer	51	12	24	45	9	20
Miscellaneous	229	42	18	206	29	14
Total	1608	307	19	1339	169	12
Normals	129	6	5			

* Deleted were all patients with allergy, arthritis, acute tuberculosis or syphilis, temp. over 99.6°F, and those with trauma, surgery or blood transfusion in past 10 days as recommended by Hall.

TABLE II. Incidence of Positive Results in the Untreated Cancer Group* According to Type or Site of Tumor.

Type or site of tumor	No. of patients	No. & % patients with positive results	
Gastro-intestinal tract	50	26	52
Cervix-uteri	31	10	32
Lung	27	13	48
Prostate	19	5	26
Leukemia	19	6	32
Breast	14	3	21
Bladder	14	5	36
Skin	6	4	67
Pancreas	9	5	56
Kidney	7	2	30
Malignant lymphoma	6	4	67
Miscellaneous	39	13	33
Total	241	96	43

* Without deletion of certain classes of patients as noted in Table I.

berculosis or syphilis, oral temperature over 99.6°F, or trauma, surgery, or blood transfusion in the previous 10 days. Omission of these patients was upon Hall's recommendation. The incidence of the type or site of the cancer is shown in Table II. The tumor was localized in 85 of the 241 cancer patients, regional node involvement or extensive local spread had occurred in 88 and distant metastases were present in 68. All of the cancers were proven by tissue examination. The normal sera were from donors accepted by the hospital blood bank. In the nonmalignant disease group were 711 men and 897 women whose average ages were 58 and 44 years respectively. The average ages of the 88 normal men and of the 41 normal women were 44 and 42 years respectively. There were 123 men and 118 women in the cancer group with respective average ages of 62 and 58 years. Most patients in the minimal disease group had psychoneurotic complaints or were to have minor surgery. Patients in the inflammation group suffered from various diseases in which the primary tissue change was one of inflammation and included those who had recently experienced surgery, accidents or burns. The majority of the patients in the heart disease group suffered from arteriosclerotic heart disease. Most patients in the hyperplasia group had benign prostatic hypertrophy. The patients in the ophthalmic disease group suffered usually from cataracts, glaucoma, or a detached retina. The most common benign tumor was uterine leiomyoma. The patients with diabetes mellitus frequently had arteriosclerotic heart disease and types of inflammation as complications. Most of the pregnancy patients were at term and delivered within the 24 hours preceding the test.

Results. Normal individuals. As shown in Table I only 5% false positive results were obtained in the group of 129 normal ambulatory persons.

Non-cancerous disease group. The incidence of positive results in this group was 19% if all non-cancerous patients were counted (Part A of Table I). This was reduced to 12% (Part B of Table I) when patients having certain disease states were deleted as recommended by Hall. The greatest reduction in false positive results produced by this maneuver (20%) was in the group of patients classified as having some type of inflammation. The effect in the other groups of deleting these patients was slight except in the pregnancy group. The highest incidence of false positive results was in the pregnancy group (39%) and the next highest in the inflammatory disease group (32%) (Part A of Table I). When the deletions recommended by Hall were made (Part B of Table I) there were no pregnancy patients and the highest incidence of false positive results was in the diabetes mellitus group (24%) followed by the gastro-duodenal ulcer group (20%). The group of patients having minimal evidence of organic disease had only 5% false positive results, a figure identical with that obtained in the group of normal ambulatory persons.

Cancerous group. As shown in Table II there were 43% correct positive results in testing the 241 cancer patients if none were deleted because of allergy, arthritis, etc. Although 2 of the various types or sites of cancer (skin and malignant lymphoma) had 67% correct positive results, they contained too few patients(6) for much importance to be attached to this high percentage figure. The next highest incidence of correct positive results was 52% in the gastrointestinal cancer

group followed by the lung cancer group with 48%. The extent of the cancerous growth did not appear to influence the incidence of positive results since 27 (32%) of the 85 patients with localized cancers had positive results, 43 (49%) of the 88 with regional spread had positive results and 26 (38%) of the 68 with distant metastases, had positive results.

Deletion from the data of non-cancerous disease patients with allergy, arthritis, acute tuberculosis or syphilis, temperature over 99.6°F and those with trauma, surgery or blood transfusion in the 10 days prior to the test, decreased the incidence of positive results in both groups by approximately the same extent, *i.e.*, 6%, thereby throwing additional doubt on the cancer specificity of the reaction.

Conclusion. (1) The Penn-Hall serofloccculation test was not found to be a satisfactory cancer selective procedure. Although it was seldom positive in normal individuals (5% of 129), it was positive in only 43% (96 of 241) of cancerous patients. Incidence of false positive results in 1608 non-cancerous patients was 19%. If patients with allergy, arthritis, acute tuberculosis or syphilis, temperature over 99.6°F and those with trauma, surgery or blood transfusion in prior 10 days were omitted as Penn and Hall suggested, the false positive results and the correct positive results were equally reduced by about 6%. (2) Positive results were more frequent among patients who had elevated blood sedimentation rates or who had inflammatory disease processes. As we evaluated, it is highly improbable that the reaction is cancer specific, a finding agreeing with the results of other investigators(3,4,5,6).

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Artifacts in Mast Cell Metachromasia Produced by Hyaluronidase Preparations.* (23795)

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In the course of recent experiments it was observed that, under specific conditions of staining with toluidine blue, mast cells of the hamster were rendered non-metachromatic by previous treatment with commercially prepared bovine testicular hyaluronidase. This finding is in direct contrast to that of other workers(3.5.15,17,18). Through a series of investigations it was possible to show that the observed effect was not the result of hyaluronidase activity but was instead an artifact.

Procedure. The tissue of the hamster studied most extensively was the cheek pouch, both in its normal state and when undergoing hyperplasia and neoplasia in response to applications of 9, 10-dimethyl-1,2-benzanthracene. Observations were also made on skin, mesentery, tongue and uterus. Tissues were fixed in Bouin's fluid, absolute ethyl alcohol and formalin, after which paraffin sections were prepared. Serial sections of each tissue were incubated 12 hours in hyaluronidase so-

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