paring the densities of glucose-free mannitol standards with standards containing known amounts of glucose, the color production by glucose can be determined and correction for glucose content of the sample applied. In our experience the density per μg of glucose is 0.0024, as compared with 0.0142 per μg of mannitol. This value for glucose remains remarkably constant with varying proportions of glucose in the sample.

The density per μg of mannitol varies within $\pm$ 2% in the range of 10 to 30 μg of mannitol. For greater accuracy or for quantities beyond these limits, a calibration curve should be prepared.

Calculation of the mannitol content of the sample is as follows:

$$\frac{D_O - D_B - (K_G \times \mu g \text{ glucose in sample})}{K_m} = \mu g \text{ of mannitol in sample.}$$

Where D_O = observed density
D_B = density of blank
K_G = density per μg of glucose
K_m = " " " " mannitol.

Summary. A simple and accurate procedure for the determination of mannitol and sorbitol based on the method of Corcoran and Page has been described.

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Hemagglutinins in the Serum of Mice of Low and High Mammary Tumor Strains.

I. DAVIDSOHN AND K. STERN.

From the Mount Sinai Medical Research Foundation and The Chicago Medical School, Chicago, Ill.

The purpose of this communication is to report striking differences in the distribution of antisheep agglutinins in the serum of mice belonging to different inbred strains.

Preparatory to studying the response of inbred mice to antigenic stimulation, it became necessary to investigate natural hemagglutinins for sheep red cells, one of the antigens used. Gorer[1] reported the presence of isoagglutinins in mice, but no reference was found in the literature to natural heteroagglutinins in mice, although many investigations dealt with such natural antibodies in other species.[2]

Material. Healthy stock animals of 4 strains were used: 100 C57 black, 73 C3H, 123 dba, and 30 Marsh-albino animals.* The sex distribution was as follows: C57 black, 59 males and 41 females; C3H, 23 males and 50 females; dba, 32 males and 91 females; Marsh-albino, 9 males and 21 females. The age of the animals ranged from 10 weeks to 20 months, with the majority from 3 to 8 months. There was no apparent relation between the agglutinating ability of the mouse serum, and the sex or age of the animals, as represented in our material.

Technic. The animals were decapitated by a rapid stroke of sharp scissors. By holding the severed parts over a funnel, the blood was caught in small test tubes. The tubes were kept slanting until the blood clotted, left in the icebox for 18 to 24 hours, and then the serum was separated from the clots. The serum was inactivated in the water bath for 30 minutes at 56°C. The titration of the antisheep agglutinins followed the method used by the senior author in previous work.[3]

1 Gorer, P. A., *Cancer Research*, 1947, **7**, 634.

2 (a) Kolle and Wassermann, *Handbuch der pathogenen Mikroorganismen*, 1929, **3**, 351, 784; (b) Shimidzu, T., *Tohoku J. Exper. Med.*, 1932, **18**, 526.

* The animals were either bred in this laboratory from parent stock received 3 years ago through the courtesy of Dr. A. Tannenbaum, Michael Reese Hospital, Chicago, or were received directly from Dr. Tannenbaum.

TABLE I.
Distribution of Sheep Agglutinins.

	Strain							
	C57 Black		C3H		dba		Marsh-albino	
Titer	No.	%	No.	%	No.	%	No.	%
0	2	2	49	67.1	55	44.7	11	36.7
1	2	2	12	16.5	23	18.7	5	16.7
2	10	10	4	5.5	23	18.7	7	23.3
4	9	9	6	8.2	9	7.4	3	10
8	19	19	2	2.7	6	4.8	3	10
	Total	42		100		94.3		96.7
16	13	13	0		2	1.7	1	3.3
32	17	17	0		4	3.2	0	
64	15	15	0		1	0.8	0	
128	5	5	0		0		0	
256	4	4	0		0		0	
512	2	2	0		0		0	
1024	2	2	0		0		0	
	Total	58				5.7		3.3
Total No. of animals	100		73		123		30	

Sheep red blood cells, at least 24 hours old and not older than one week, were washed 3 times by centrifuging. The cells were not used unless the last supernatant was colorless. From the packed sheep cells a 2% suspension in physiologic solution of sodium chloride was prepared.

The test was set up as follows: Two drops of physiologic saline were added to each tube except the first. Two drops of serum were added to the first and second tube. After mixing, 2 drops of the second tube were transferred to the third tube, and so on. This gave serum dilutions of 1:2, 1:4, etc., which were continued according to the height of titer expected. One drop of the 2% sheep cell suspension was added to each tube and the rack was shaken. The tubes were incubated at room temperature for 2 to 3 hours. The reading of the agglutination was done grossly, and checked microscopically. Heavy "buttons" breaking up in large fragments were read as 3 plus; "buttons" breaking up in smaller grossly visible fragments, 2 plus; fine clumps visible under low-power magnification, 1 plus. The titer was given as the reciprocal of the highest dilution in which agglutination was found.

Results. Table I summarizes the titers obtained in the 4 inbred strains. Sheep agglutinin titers in C57 blacks reached by far higher values than those observed in any of the other strains. 58% of the C57 blacks presented titers of 16 or more, while among the C3H none of the titers exceeded 8; less than 6% of the dba animals and about 3% of the Marsh-albino exceeded this limit. 1024 was the highest titer observed in the C57 blacks; the highest titers in the C3H, dba and the Marsh-albino strains were 8, 64, and 16, respectively.

On the other hand, failure to agglutinate sheep erythrocytes in undiluted serum was found in only 2% of the C57 blacks, whereas 67.1% of C3H mice, 44.7% of dba animals, and 36.7% of Marsh-albinos showed titers of 0.

In order to study some of the properties of the antisheep agglutinins, pools of serum were prepared by sacrificing 9 to 11 animals of the C57 black strain. Preservation of the serum at 3-8°C for one month showed only insignificant changes in the agglutinin titer; after two months, usually a drop of the titer by 2 or more tubes was observed.

[3] Davidsohn, I., *J.A.M.A.*, 1937, **108**, 289.

TABLE II.
Absorption Experiments.

	Titer		
		After absorption with	
	Unabsorbed serum	Guinea pig kidney	Beef cells
Pool I	32	20	20
" II	64	40	40
" III	64	40	40

Table II gives the results of absorption of the serum with suspensions of guinea-pig kidney and of beef erythrocytes. The preparation of these antigens followed the technic given by the senior author.[4] 0.1 cc of the serum was mixed with 0.5 cc of the antigen suspension, and the mixture was kept for 1 hour at room temperature, with repeated shaking of the mixture. Following centrifugation, the supernatant was set up simultaneously with the unabsorbed serum. For the calculation, the dilution of the serum during the absorption was taken into consideration. Neither guinea-pig kidney nor beef red cells proved capable of removing the agglutinins from the serums to an appreciable degree.

Reading of the agglutination after increasing time intervals showed in some instances slightly higher titers after 2 hours of incubation as compared with the one hour titers; after 2 hours, the titers did not rise any further during observation for 24 hours. No significant change in titers was noted when the same serums were incubated at room temperature or 37°C. Occasionally, prolonged ice box incubation increased the titer by 1 or 2 tubes.

Instead of physiologic saline solution, albumin solution (20% bovine albumin) or serums of mouse, rat and rabbit were used as diluents for the serum to be tested and for the sheep cells. In the case of the animal serums, previous tests had shown the absence of antisheep agglutinins, or the serums were absorbed with sheep cells prior to use. In none of these experiments, did albumin or serum diluents raise the titer found with saline diluent; quite frequently, the titers in the albumin or serum dilutions were 2 to 3 tubes lower than the saline titers.

Table III lists agglutination tests which were set up with human O Rh-positive blood cells. The serum of 57 C57 blacks, of 61 C3H mice, and of 13 dba animals was tested. In marked contrast to the results obtained with sheep agglutinins, human red blood cells were clumped by less than 25 per cent of mouse serums and only in low titers. No appreciable difference was found between serums derived from animals of different strains. Nor was there any relationship between the simultaneously determined titer of sheep agglutinins, and the presence or absence of agglutinins for human erythrocytes.

In a few instances, also the agglutination of human erythrocytes of groups A and B (both Rh-positive) was tested with the serum of C57 black and of C3H mice. The number of experiments is too small to permit any conclusion. A cells showed a tendency to be clumped at a higher titer than O cells, but also here the titers were frequently far below those found in the same serum for sheep agglutinins.

Animals with spontaneous, transplanted and induced tumors were studied for the presence of hemagglutinins. The occurrence of antisheep agglutinins was not essentially different in tumor-bearing animals as compared with tumor-free animals of the same strains. A sufficiently large material to warrant definite conclusions is under investigation and will form the subject of a separate report.

Discussion. In Table IV the distribution of antisheep agglutinins is compared with the

TABLE III.
Agglutination of Human O Rh-positive Blood Cells By Mouse Serums.

	Strain		
Titer	C57 Blacks	C3H	dba
0	44	47	10
1	7	10	3
2	2	3	0
4	1	1	0
8	2	0	0
16	1	0	0
Total No. of Animals	57	61	13

[4] Davidsohn, I., and Walker, P. H., *Am. J. Clin. Path.*, 1935, **5**, 455.

TABLE IV.
Antisheep Agglutinins and the Incidence of Spontaneous Mammary Cancer in Inbred Strains of Mice.*

Strain	Authors	Tumor incidence, %	Antisheep agglutinins	
			Absent %	16 and higher, %
C3H	Andervont ('41)	91.4	67.1	0
	Bittner ('43)	92.3		
dba	Korteweg ('36)	76.3	44.7	5.7
	Murray & Hoffman ('41)	64.5		
Marsh-albino	Murray & Hoffman ('41)	76.4	36.7	3.3
	Haagensen & Randall ('42)	76.4		
C57 Black	Little, Murray, & Cloudman ('39)	0.5	2.0	58.0
	Haagensen & Randall ('42)	1.1		

* First 3 columns adapted from Walter E. Heston: Genetics of Mammary Tumors in Mice, in "A Symposium on Mammary Tumors in Mice," AAAS, 1945; p. 61, table I.

incidence of spontaneous mammary carcinoma, as recorded in the literature for the mouse strains furnishing the material of this study. It is apparent that the incidence of spontaneous mammary carcinoma in females in these strains is roughly inversely proportional to the presence and titers of the antisheep agglutinins. For instance, mice of the C57 black strain with extremely low mammary cancer incidence showed a very low percentage of serums lacking in sheep agglutinins and a high percentage of serums with titers of 16 and more. On the other hand, more than two-thirds of the serums of C3H mice, which possess the highest rate of tumor incidence, were completely lacking in agglutinins, and none of the animals so far examined showed a titer higher than 8. Animals of the dba strain, which develop mammary cancer quite frequently but less frequently than the C3H mice, lack sheep agglutinins in almost one-half of the cases, and less than 6% of the animals had titers of more than 8. Marsh-albinos, of which only a rather small number was tested, occupy a similar position in regard to tumor incidence; no agglutinins were found in more than one-third of the cases, and only 3.3% had titers higher than 8.

While no definite conclusions can be drawn from these findings at present, it may be hypothetically assumed that they are not coincidental but that the presence and the titer of the hemagglutinins are in some way connected with the genetic and other factors characterizing these mouse strains. In view of the fact that the incidence of spontaneous mammary carcinoma in the strains under discussion is determined by the milk factor and by hormonal influences in addition to genetic factors, those factors will have to be investigated.

The experiments on absorption of the agglutinins with guinea pig kidney suspensions showed that the agglutinins are not of the Forssman type; they also differed from the agglutinins found in serum of patients with infectious mononucleosis, since these latter are absorbed by beef red cells.[5] In this connection it may be pointed out that the position of the mouse in the Forssman system is not fully clarified. While older investigations placed this species into the "guinea pig group"[6] other workers presented contradictory evidence,[7] and more recent work showed differences between the true Forssman antigen and the antigens present in mouse tissues.[8] It is likely that especially the older investigations were carried out with heterozygous animals. For this reason studies on this

[5] Davidsohn, I., *Am. J. Clin. Path.*, 1938, **8**, 56.

[6] Doerr and Pick, *Biochem. Z.*, 1913, **50**, 129 (quot. 2a).

[7] Davidsohn, I., *Arch. Path. and Lab. Med.*, 1927, **4**, 776.

[8] Brown, G. C., *J. Immunol.*, 1943, **46**, 325.

problem are being carried out in this laboratory using inbred strains.

Previous work on the carmine storage in mice of inbred strains[9] demonstrated an inferior reticulo-endothelial storing ability in animals of the C3H strain as compared with C57 blacks. Although the origin and the significance of natural antibodies, such as the antisheep agglutinins studied in the present report, is not yet understood, there is considerable evidence in support of the part played by the reticulo-endothelial system in their production. In addition to the previously reported depression of the storing capacity of reticulo-endothelial tissues, the present study suggests impairment of the reticulo-endothelial system in relation to the production of natural antibodies in some mouse strains with high spontaneous mammary cancer incidence as compared with the resistant C57 black strain.

Summary. Heteroagglutinins for sheep erythrocytes were studied in the serum of 4 inbred mouse strains. Antisheep agglutinins were present much more frequently and in significantly higher titers in C57 black than in C3H, dba, and Marsh-albino strains. Some properties of the sheep agglutinins were studied. No such differences were found in the distribution of heteroagglutinins for human red cells in the different strains. The possible significance of these findings was discussed.

9 Stern, K., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 315.

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Nature and Probable Origin of Conjugated Histamine Excreted After Ingestion of Histamine.

KARL F. URBACH. (Introduced by Carl A. Dragstedt.)

From the Department of Pharmacology, Northwestern University Medical School, Chicago, Ill.

Anrep and coworkers reported that a histamine derivative appeared in the urine of various animals and a human subject after oral administration of histamine. This histamine derivative was inactive when tested on the isolated guinea pig ileum, but after acid hydrolysis of the compound a typical histamine response could be elicited. Injection of histamine was not followed by increased excretion of the derivative. Liver or intestine were suggested as likely sites for the conjugation of the ingested histamine.[1] Rosenthal and Tabor recently made the important observation that the chemical behavior of the urinary histamine derivative resembled that of acetyl histamine [4(β-acetyl aminoethyl) imidazole].[2]

The purpose of the present investigation was to test whether or not the histamine derivative is actually identical with acetyl histamine and to discover the origin of the compound in the body. The experiments described below present evidence, obtained by paper chromatography, for the identity of the histamine derivative with acetyl histamine and show that this compound appears in the feces as well as in the urine after oral administration of histamine. It is further shown that histamine is rapidly acetylated when added to fresh but not when added to previously autoclaved feces. Lastly, some common fecal microorganisms are shown to produce traces of acetyl histamine from histamine. The evidence obtained suggests that ingested histamine is acetylated within the intestinal contents from which it is partially absorbed and excreted by the kidneys. The methods employed are modifications of those previously developed for the identification of histamine

1 Anrep, G. V., Ayadi, M. S., Barsoum, G. S., Smith, J. R., and Talaat, M. M., *J. Physiol.*, 1944, **103**, 155.

2 Rosenthal, S. M., and Tabor, H., *J. Pharm. and Exp. Therap.*, 1948, **92**, 425.