before mentioned were 5.42 hours and 8.07 hours—a variation of only 2.65 hours. No correlation was noticed as to size or breed.

The results reported in this paper are higher than those obtained by Ivy[1] and by Thomas.[2] The former reports the normal emptying time of the stomach in 5 dogs as varying from 4 hours and 28 minutes to 5 hours and 17 minutes. He used a meal of 125 gm. of Swift's Silver Fox Food and 100 cc. of milk. Thomas states that a dog fed about 300 gm. of dog biscuit and a pint of milk would probably empty in somewhere around 3 hours. Both of these authors used a different type of meal from the one reported in this paper and this probably accounts for the difference in results. The experimental meal we used proved very successful in our work and we can recommend it highly; it is fairly well balanced; the dogs ate it with relish and small dogs subsisted solely on it.

Conclusions. The normal emptying time of the stomach was determined on 25 dogs under carefully controlled conditions. The meal consisted of 40 gm. of hamburger steak, 10 gm. of dried ground bread and 50 cc. of milk; barium sulphate was added (15 gm.). A total of 200 tests were made. The average emptying time of the stomach of the dogs was 6.59 hours; the average for the tests was 6.61 hours. The variations were between 5.42 and 8.07 hours. It was found that the emptying time of the stomach for an individual dog was strikingly uniform from day to day.

7007

Effect of Massage on Blood Platelet Production.*

S. P. LUCIA AND J. F. RICKARD. (Introduced by Wm. J. Kerr.)

From the Department of Medicine, University of California Medical School, San Francisco.

Fenn[1] demonstrated the ease with which erythrocytes undergo hemolysis when they come in contact with glass. Davidson[2] showed that a relatively large proportion of erythrocytes contain basophilic staining-substance. Watson[3] concluded that "the fragments of

[1] Ivy, A. C., and Fauley, G. B., *Am. J. Physiol.*, 1929, **91**, 206.

[2] Thomas, J. E., *J. Am. Med. Assn.*, 1931, **97**, 1663.

* Assisted by a grant from the Christine Breon Fund.

[1] Fenn, W. O., *J. Exp. Med.*, 1922, **35**, 271.

[2] Davidson, L. S. P., *Edinburgh Med. J.*, 1930, **37**, 425.

[3] Watson, C. H., *Edinburgh Med. J.*, 1932, **39**, 229.

effete or damaged erythrocytes correspond in every detail to the bodies known as blood platelets." For these reasons, we wish to record our observations on the blood platelets of the rabbit. During these experiments it was observed that there were marked variations in the platelet-counts from the same animals, and that the estimated counts from blood-smears did not agree with those calculated in the counting-chamber.

The blood platelets of normal adult rabbits were counted. Chamber-counts and smears were prepared from the same drop of blood obtained from the ear (a) without trauma, (b) after moderate trauma, and (c) after prolonged trauma. Variations between chamber- and smear-counts were studied, and the effects of time on the formation of platelets in the pipette were observed. The massage consisted of gentle, but firm, stroking of the ear at a rate of 25 strokes per minute for one minute and for 5 minute periods. A distal venous ramus of the ear was punctured and the first drop of blood withdrawn was studied. Anders Kristenson[4] fluid was used to dilute the blood. It was filtered and centrifugalized for 20 minutes immediately before each experiment. An erythrocyte-counting pipette was filled as follows: diluent to the 0.5 mark; blood to the 0.5 mark; then diluent to the 101 mark. The pipette was shaken for 3 minutes and a Levi-Neubauer counting-chamber was filled and allowed to stand for 20 minutes. Only smooth, rounded, highly refractile bodies were counted as platelets. The limit of accuracy by this method was 70,000 per cu. mm. Platelets were classified as "large" when 4 micra or greater, and "small" when less than 4 micra, in diameter. The large platelets appeared as clear, rounded, or ovoid bodies containing dark-staining, highly refractile granules. The smear stained by Wright's method was divided into 9 equal zones and the number of platelets per 1,000 erythrocytes in each zone estimated. The average for 9,000 red blood cells was multiplied by the total erythrocyte count expressed in thousands, in order to obtain the total platelet-count per cu. mm. of blood.

The following tabulation records a typical observation on a series of 5 rabbits. The figures express the average platelet count per cu. mm. of blood:

Effects of Massage on Platelet-Count

	Normal	After 1 min. Massage	After 5 min Massage
Large	342,000	338,000	286,000
Small	540,000	906,000	434,000
Total	882,000	1,244,000	720,000

[4] Kristenson, Anders, *Acta. Med. Scand.*, 1922-23, **57**, 301.

To show the discrepancy between chamber- and smear-estimation platelet counts, the pipettes were filled and the smears made from the same drop of blood. The following gives the average values for 13 such counts:

The Difference between Chamber- and Smear-Counts

Chamber-Count	Smear-Estimation
927,000	329,000

A third experiment demonstrates the effect of time on successive drops withdrawn from the same pipette at varying intervals of time. The pipette was thoroughly shaken before each successive count:

Effect of Time on Platelet-Count (Chamber-Method) from same Pipette.

	Normal	After ¾ hr.	After 1½ hrs.	After 4 hrs.
Large	200,000	240,000	270,000	190,000
Small	580,000	1,040,000	950,000	560,000
Total	780,000	1,280,000	1,220,000	750,000

Similar observations have been repeatedly made on the same and different animals. They vary only in the degree of initial platelet count, the general trend of each experiment being unaffected.

To control any variation in the platelet counts due to excitement, an animal was handled in the same manner as in the previous experiments, except that the ears were not traumatized. Platelet counts done before and after a one-minute interval do not show any significant variation.

Effect of Excitement on Platelet-Count

	Normal	One minute later without trauma
Large	170,000	100,000
Small	270,000	360,000
Total	440,000	460,000

To determine whether the change in platelet count of the traumatized ear was the reflection of a local or a general systemic change, a platelet count was done on blood obtained from an untraumatized ear. After one minute of trauma, platelet counts were made on blood obtained from the traumatized and opposite, untraumatized ears. The results follow:

Effect of Trauma on Platelet-Count of Opposite Ear

	Right ear before massage	Right ear after massage	Untraumatized left ear
Large	85,000	230,000	85,000
Small	360,000	460,000	218,000
Total	445,000	690,000	303,000

Summary. Moderate massage or trauma causes an increase in the total platelet count. After prolonged trauma, the count drops to normal. These phenomena occur locally in the traumatized ear. The counting chamber method of enumerating blood platelets gives values which are 2.8 greater than the smear estimation values. The platelet count, from the same pipette after increasing intervals of time, rises at first and then falls. Anders Kristenson fluid is undesirable for counting blood platelets because it dissolves erythrocytes.

7008 P

The Fixation of Certain Viruses on the Cells of Susceptible Animals and Protection Afforded by Such Cells.

PEYTON ROUS, PHILIP D. MC MASTER AND STEPHEN S. HUDACK.

From the Rockefeller Institute for Medical Research, New York City.

Rabbit embryo tissue was grown in a thin plasma clot and after some days the cultures were submitted to a trypsin solution strong enough to digest the clot and free the cells that had extended into it.[1] By repeated pipettings and filtrations through lens paper, alternated with differential washings in gelatin-Tyrode solution, suspensions were obtained of the living cells as individuals. They were mixed at room temperature with suspensions of virus, and after an interval the cells were recovered with the centrifuge, again washed repeatedly, treated in various ways and inoculated into rabbits. The viruses used were vaccinia and the filterable agent causing the rabbit fibroma described by Shope.[2]

Cells exposed to virus and repeatedly washed invariably gave rise to lesions in susceptible animals. The briefest exposure at room temperature permitted by the conditions resulted in an association of the virus with the cells, which withstood many washings of the latter. The fixation thus indicated took place not only upon living cells but upon those killed by heat, ultraviolet light, and water respectively, and often was as considerable. Incubation of the material with immune serum *in vitro,* followed by repeated washings prior to infection, resulted in neutralization of the virus associated with dead cells, whereas these procedures were without effect when

[1] Rous, Peyton, and Jones, F. S., *J. Exp. Med.,* 1916, **23**, 549.

[2] Shope, R. E., *J. Exp. Med.,* 1932, **56**, 793.