

THE RATE OF DIGESTION OF HUMAN BLOOD BY CERTAIN SPECIES OF MOSQUITOES

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Summary

The rate of digestion of human blood was investigated at 4-hourly intervals by the precipitin and benzidine tests for the mosquito species *Aedes aegypti*, *A. concolor*, *A. notoscriptus*, *A. scutellaris*, and *Culex fatigans*. In an environment in which temperature and humidity were constant and in which there were equal periods of light and darkness, the rate of digestion ranged from 31 hr for *A. scutellaris* to 48 hr for *A. concolor*.

Increasing the proportion of darkness during the test period increased the rate of digestion in *A. notoscriptus*.

In experiments with *A. aegypti* and *A. scutellaris* the rate of digestion was similar in females 1 and 3 weeks old.

The benzidine test showed that haematin was defaecated for up to 90 hr after a blood meal.

I. INTRODUCTION

The "rate of digestion"† of blood by a female mosquito may affect its efficiency as a vector of diseases, since a female which digests its blood meal rapidly may feed at more frequent intervals than one which takes longer to digest its meal. It is of some value to know the rate of digestion for other purposes also; for example, the period of time during which the precipitin test can determine the origin of the host's blood is dependent upon the rate of digestion of the blood.

The precipitin test has been used previously to determine the arbitrary end-point of digestion of the blood serum proteins (reviewed by Bates 1949; West and Eligh 1952; Weitz and Buxton 1953) but these determinations were insufficiently critical to show finer differences in the rate of digestion by various species of mosquitoes. The benzidine test, however, has not been used previously in such studies and, although certain precautions are necessary in executing and interpreting it, the test was used in preference to others because it is so sensitive in detecting minute amounts of iron porphyrins.

The time required for the digestion of blood has been shown by Shlenova (1938) and West and Eligh (1952) to depend upon temperature and to a lesser extent upon humidity, but the effect of the period of illumination upon this process does not seem to have been studied. Therefore, the rate of digestion for *Aedes notoscriptus* was investigated under various conditions of light and darkness to determine whether this rate was affected by variation of these conditions.

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† The "rate of digestion" herein refers to the period of time required for blood meals of various mosquito species to reach such a stage of digestion that the serum proteins no longer precipitate their specific antibodies.

II. METHODS

The mosquitoes used were females of *A. aegypti*, *A. concolor*, *A. notoscriptus*, *A. scutellaris*, and *Culex fatigans* which had not fed previously. Those which fed on human blood were transferred to 4-in. gauze cages without food or water, and were kept in an incubator with a glass door. The temperature of the incubator was $27 \pm 1^\circ\text{C}$, and the saturation deficit was 2 ± 1 mm Hg. Groups of the fed adults were killed at 4-hourly intervals, smeared whole onto filter papers (Eligh 1952), and kept dry in a refrigerator until the precipitin and benzidine tests could be applied to them.

TABLE 1

THE RATE OF DIGESTION OF HUMAN BLOOD BY VARIOUS SPECIES AS DETERMINED BY THE PRECIPITIN TEST

In this and subsequent tables, figures show the percentage of mosquitoes which gave positive reactions for either the precipitin test or the benzidine test, whilst the figures in brackets show the number of mosquitoes tested

Mosquito Species	Percentages and Numbers of Mosquitoes (in brackets) giving Positive Precipitin Tests								50% Rate of Digestion (hr)
	Time after Ingestion of a Blood Meal (hr)								
	24	28	32	36	40	44	48	52	
<i>A. scutellaris</i>	100(8)	100(21)	42(57)	0(15)	0(4)	0(11)	0(21)	0(7)	31
<i>A. notoscriptus</i>	100(20)	100(18)	100(59)	70(42)	28(57)	0(20)	0(15)	0(7)	36
<i>A. aegypti</i>		100(20)	100(25)	51(43)	0(17)	0(14)			38
<i>C. fatigans</i>			100(8)	100(15)	100(27)	60(30)	34(26)	0(15)	46
<i>A. concolor</i>					100(7)	100(12)	34(15)	0(6)	48

The influence of the ratio of light to darkness upon the rate of digestion of *A. notoscriptus* was determined as follows. After having been fed, the mosquitoes were divided into five lots, one lot being kept in each of the following conditions for 36 or 40 hr: constant darkness, constant light, light to darkness ratios of 2:1, 1:1, and 1:2. In the dark period, the glass door of the incubator was blacked out. In the light period, a 40-W incandescent tube was placed 9 in. in front of the glass door. At the end of the 36- or 40-hr period the mosquitoes were killed and smeared onto filter papers, which were then checked by the precipitin test for the presence of undigested serum proteins.

The process of digestion of blood by *C. fatigans* was observed at 12-hourly intervals and correlated with the rate of digestion which had been determined by the precipitin test.

In performing the precipitin tests, the filter paper smears were extracted with physiological saline, the extracts were run up small bore capillary tubes, and the specific antisera (prepared from laboratory animals after the method of Proom 1943) were run up the capillary tube beneath them. The details of this test were given in a previous paper (Lee, Clinton, and O'Gower 1954).

Blood smears and defaecated matter collected on the bottoms of the 4-in. gauze cages (after the digestion of the serum proteins), were checked for the presence of iron porphyrins by applying the benzidine test to them, after they had been heated in a steam oven for 10 min at 110°C. The benzidine test was checked for specificity in detecting the iron porphyrins in the mosquito abdomen by testing unfed, sugar-fed, and blood-fed adults of *A. scutellaris*. Only those mosquitoes which had fed on blood gave positive reactions.

III. RESULTS

At constant temperature and humidity and when the ratio of light to darkness was 1:1, there were differences in the rates of digestion of human blood for the five mosquito species tested (Table 1). By interpolation, the rates of digestion of 50 per cent. of the population varied from 31 hr for *A. scutellaris* to 48 hr for *A. concolor*.

From visual observations of *C. fatigans* at 12-hourly intervals, the progress of digestion was as follows: immediately after the ingestion of a blood meal the female mosquitoes were fully engorged with red blood; this turned black within 6 hr. At

TABLE 2

THE INFLUENCE OF THE RATIO OF LIGHT TO DARKNESS UPON THE RATE OF DIGESTION OF HUMAN BLOOD BY *A. NOTOSCRIPATUS*

Time (hr)	Percentages and Numbers of Mosquitoes (in brackets) giving Positive Precipitin Tests				
	Constant Illumination	Light to Darkness Ratio 2:1	Light to Darkness Ratio 1:1	Light to Darkness Ratio 1:2	Constant Darkness
36	100(27)	100(30)	70(42)	54(48)	46(54)
40	40(50)	33(83)	28(57)	37(60)	0(25)

12 hr, the abdomen was slightly more than three-quarters distended with blood. At 24 hr, the distended abdomen was about two-thirds filled with blood. After 36 hr, only the anterior half of the distended abdomen was filled with blood. At 48 hr, slightly less than half of the abdomen was distended with blood. Sixty hr after the meal, the abdomen was less than one-third distended with the breakdown products of digestion. During this period the distention of the abdomen gradually decreased, until at 60 hr it had the appearance of a gravid mosquito.

At constant humidity and temperature, and when the ratio of light to darkness varied in a given period (the critical digestion period in Table 1 (i.e. 36-40 hr)) from constant illumination to constant darkness, the rate of digestion of *A. notoscriptus* increased when the proportion of darkness in each period was increased (Table 2).

Positive reactions for the benzidine test were obtained up to 90 hr after ingestion, and, as defaecated matter collected on the bottom of the cages after the digestion of the blood meal also gave positive reactions, it was concluded that the haem of the haemoglobin was undigested and defaecated as a haematin-like substance.

IV. DISCUSSION

The progress of digestion of blood meals by mosquitoes has been studied by several methods. These include the precipitin test used by Bull and King (1923), the histological method of Huff (1934), the observational studies of Shlenova (1938) and Hosking and MacInnes (1948), the crystallization method of Biocca (1950), and the studies on proteolytic digestion of blood by Fisk and Shambaugh (1952).

The benzidine test is non-specific, but it is extremely sensitive for the detection of iron porphyrins, of which haematin, a product of certain types of haemoglobin digestion, is one. From the results of this investigation it was apparent that iron porphyrins were present in the mosquito abdomen long after the serum proteins had been digested, and presumably also after the digestion of the globin moiety of haemoglobin. As defaecated matter also gave the same reaction, it was deduced that a haematin-like substance was defaecated rather than digested. This is in keeping with West and Eligh's finding (1952) with mosquitoes using the Meyer reduced phenolphthalein test. Wigglesworth (1943) obtained similar results when investigating the digestion of blood by *Rhodnius prolixus*.

There was no difference in the rate of digestion of 1- and 3-week-old adult mosquitoes; thus the use of a population in which the ages of the females varied had no effect upon the rates obtained.

Forty-eight hr after the ingestion of a blood meal by *C. fatigans* the precipitin test no longer gave a positive reaction, and, from visual observations, this time corresponded to the stage in digestion when the abdomen was slightly less than half distended, and it was then that the practical limitation of the precipitin test was reached in identifying the blood meal source.

It has been shown by previous authors that the rate of digestion of blood increases with rising temperature (Shlenova 1938; West and Eligh 1952) and with increasing humidity at low temperature (Shlenova 1938). The experiments recorded here show that it also increases as the ratio of dark to light increases for the species *A. notoscriptus*. However, as the general activity rhythms of all mosquito species would not be the same under similar conditions of illumination, it is not possible to generalize from this observation.

The rate of digestion of blood shows a wide range of specific variations which appear small when the measurements are made at 24-hr intervals (West and Eligh 1952). By using 4-hourly intervals between determinations, these differences become more obvious. Although Hovanitz (1947) showed that "the number of *Plasmodium gallinaceum* cysts" ingested by the adults of *A. aegypti* in a blood meal varied according to the size of the adult, such an explanation could hardly be given for these specific differences in the rates of digestion, since the intraspecific variation in the size of the adults was almost as great as the interspecific variation. Thus the different rates of

digestion of human blood by the species of mosquitoes tested would seem to be due to specific differences in the digestive processes and not to specific differences in the size of the adults.

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