feed to the same extent as in Nature. While a blood feed will lead to egg formation in virgin *A. stephensi* and *A. annularis*, it fails to do so in virgin *A. subpictus*, even when this mosquito has been allowed to feed to repletion on man, goat, or cattle, and evidence of follicular stimulation is the only noticeable change. This led Christophers¹ to abandon his observations on egg development in *A. subpictus* in the laboratory.

The presence of two distinct phases in the egg formation in *Aedes aegypti*, one stimulation of the follicle and the other formation of the ovum, was tentatively suggested by me². The stage of follicular stimulation can be clearly demonstrated in *A. subpictus*, and is characterized by the appearance of very light peppery granules scattered irregularly. These are distinct from yolk granules, which are much coarser and are deposited in a much more compact manner. Distinction between these two stages in *A. annularis* is not possible unless the latter are allowed to ingest a very small quantity of blood, as otherwise the second phase overlaps the first.

It has also been observed in *A. subpictus* caught in Nature that ingestion of a large quantity of blood and yolk formation are solely dependent on the presence of sperms in the theca.

While reception of blood into the stomach is sufficient to cause stimulation of the follicles and yolk formation in virgin *A. annularis*, and only the former change in virgin *A. subpictus*, the introduction of sperms into the theca in the latter species can alone lead to yolk formation. The specific substance of the blood which is concerned with yolk formation in *A. subpictus* is intimately associated with the stimulation of the theca, and it is presumed a specific hormone is thereby produced.

Any suggestion that the failure of laboratory-fed *A. subpictus* to produce yolk owing to the ingestion of a smaller quantity of blood than the minimum amount necessary for egg production is untenable on account of the constant association between yolk formation and the presence of sperms in the theca, and also the reverse phenomenon noticed in mosquitoes caught in Nature². Besides, the amount of blood ingested by *A. subpictus* in the laboratory does not seem to be insufficient for egg production.

Collections of the mosquitoes, *A. annularis* and *A. subpictus*, were made from the salt-lake area adjacent to Calcutta, and dissections were performed from day to day. Nearly five hundred specimens of each species were dissected.

The above conclusions have been arrived at from observations made during the winter months.

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¹ Christophers, S. R., Paludism. Government Central Branch Press, Simla (1911).

² Roy, D. N., *Bull. Ent. Res.*, 27, 423-429 (1936).

Photodynamic Hæmolysis by 3 : 4-Benzpyrene

IN a recent communication, Wolman¹ described the photodynamic hæmolysis of sheep's red corpuscles by carcinogenic hydrocarbons. Though unable to demonstrate this in 1937, we did succeed last year using long exposures to light. Our results agree

closely with Wolman's. But we varied and further extended the experiments in order to try to see how benzpyrene sensitizes red corpuscles. We have carried out the following investigations.

(1) Colloid solutions of benzpyrene in normal saline were made up in a series of dilutions from 1 in 10,000 to 1 in 100 million. Washed human red corpuscles were added to these and the mixtures kept in the dark for three hours. Some were then put in sunlight and controls kept in the dark. After 45 minutes exposure the red corpuscles in the 1 in 10,000, 1 in 100,000, and 1 in 1 million solutions showed approximately the same amount of partial hæmolysis. This was complete in two hours, by which time a partial hæmolysis was present in the 1 in 10 million solution. There was no hæmolysis in the 1 in 100 million solution in the light and none in the controls kept in the dark.

(2) Colloid benzpyrene solutions were given a prolonged exposure to light. Red corpuscles were then added and the mixtures kept in the dark. No hæmolysis resulted.

(3) Suspensions of red corpuscles in benzpyrene colloid were left in the dark for various times and the corpuscles then washed free from benzpyrene. After this they were given a long exposure to light. Corpuscles which had been in contact with the hydrocarbon for five minutes showed a trace of hæmolysis, those in contact for one hour showed moderate hæmolysis, and corpuscles which had been in contact with the benzpyrene for three hours showed complete hæmolysis, in 45 minutes exposure to sunlight. The strength of the colloid was 1 in 100,000.

(4) Samples of red corpuscle suspensions in benzpyrene colloid (1 in 250,000) kept for 2½ hours in the dark at various temperatures were washed and exposed to light. Those which had been kept in contact with the benzpyrene at 0° C. showed only slight hæmolysis after prolonged exposure to a mercury vapour lamp. Red corpuscles which had been in contact at 37° C. showed a comparatively rapid hæmolysis under the lamp.

Thus, using red corpuscles, the photodynamic potency of benzpyrene is roughly the same in dilutions of 1 in 10,000 to 1 in 1 million. *Paramecia* gave a similar result (Doniach²). Washing red corpuscles which have been suspended in benzpyrene does not get rid of their sensitivity to light. This sensitivity is enhanced by prolonged contact with the hydrocarbon and by raising the temperature at which the corpuscles are suspended in the colloid. These results indicate that the benzpyrene must enter into the red corpuscles before a photodynamic action can start. If it were a matter of simple adsorption on to their surface one would have expected contact at 0° C. to have been more effective than contact at 37° C. The enhancement at higher temperature is likely to be due to an increased solubility of the benzpyrene in water, an assumption in accord with recent results of other experiments in this laboratory.

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¹ Wolman, M., *NATURE*, 145, 592 (1940).

² Doniach, I., *Brit. J. Exp. Path.*, 20, 227 (1939).