sieren (TELLUR); als folgendes haben wir also das Gold eingereiht. Dabei sei aber betont, dass es mit unserer Methode gelingt submikroskopische Quantitäten aufzudecken, sodass wir darin über ein sehr subtiles Mittel verfügen das Schicksal des Goldes zu verfolgen und zwar in tadellosen histologischen Praeparaten, die ohne weiteres eine ganze Reihe von Färbungen und damit von Strukturuntersuchungen zulassen.

Eine vollständige Beschreibung der Befunde und Methodik wird in der Zeitschrift für mikroskopisch-anatomische Forschung erscheinen.

Physiology. — *Cellophan an Stelle von Kymographionpapier.* Von FRIEDRICH KRÜGER. (Aus dem Institut für vergleichende Physiologie der Reichsuniversität Utrecht.) (Communicated by Prof. H. J. JORDAN.)

(Communicated at the meeting of January 30, 1937).

Das Cellophan erfreut sich für wissenschaftliche Zwecke eines stets wachsenden Anwendungsgebietes. Im Folgenden möchte ich auf die Verwendbarkeit der Glashaut — wie das Cellophan auch genannt wird — zum Aufzeichnen von Kurven auf der Kymographiontrommel hinweisen. Da das Cellophan vollkommen durchsichtig ist, ist es möglich, die so gewonnenen Kurven ohne weiteres mit dem Projektionsapparat zu demonstrieren, ohne dass man gezwungen ist, dem Umweg über die photographische Platte oder dergl. einzuschlagen. Ausserdem ist es möglich, durch photographischen Kontaktabdruck die Kurven beliebig zu vervielfältigen. Als besonderer Vorteil fällt noch die Billigkeit des Cellophans ins Gewicht.

Das Aufziehen des Cellophans auf die Kymographiontrommel erfolgt in der Weise, dass man ein entsprechend grosses Stück mit Wasser gründlich durchfeuchtet, wobei es nicht darauf ankommt, dass das Stück zerknittert wird. Dann legt man das noch feuchte Cellophan möglichst glatt auf die Trommel auf. Grössere Falten und alle Luftblasen streicht man mit Hilfe eines feuchten Schwammes aus. Ein besonderes Festkleben des freien Endes ist nicht notwendig, da es bei dem nun folgenden Trocknen ohne weiteres auf dem unterliegenden Cellophan haften bleibt. Beim Trocknen verkürzt sich das Cellophan, zieht sich zusammen und spannt sich auf diese Weise ausserordentlich glatt auf die Unterlage, sodass alle noch vorhandenen kleineren Falten verschwinden. Ist letzteres erreicht, muss man berussen, ehe die Membran vollkommen getrocknet ist. Wartet man bis zum völligen Trocknen, so verkürzt sich die Haut unter der Einwirkung der Hitze so stark, dass sich das angeklebte Ende wieder ablöst, was natürlich vermieden werden muss. Das Berussen erfolgt in der gewohnten Weise. Es empfiehlt sich hierbei die Klebestelle zu markieren, da diese

unter der Russschicht wegen der Dünne des Materials vollkommen unsichtbar wird.

Ist die Trommel beschrieben, so lässt sich das Cellophan von der Klebestelle beginnend leicht abnehmen, ohne dass es nötig ist, die Membran zu zerschneiden. Die Kurven können dann in gewohnter Weise in Schellacklösung fixiert werden. Man benutzt allerdings eine dünnere Lösung als bei Papier, da andernfalls die eintrocknende und sich dabei verkürzende Schellackschicht Falten im Cellophan hervorruft.

Zur Herstellung der Diapositive ist es nur nötig, die Kurvenstücke zwischen Glasplatten aufzustellen. Die Einfachheit und Billigkeit des Verfahrens wird es in geeigneten Fällen von Nutzen erscheinen lassen.

Medicine. — *Some observations on the salivary and stomach secretion of Anopheles and other mosquitoes.* By A. DE BUCK. (From SWELLENGREBEL's Zoological Laboratory in the Department of Tropical Hygiene of the Royal Colonial Institute at Amsterdam). (Communicated by Prof. W. A. P. SCHÜFFNER).

(Communicated at the meeting of January 30, 1937).

The difference of the staining reactions in the median and lateral lobes of the salivary glands of *Anopheles*, well known since the investigations of GRASSI, SCHÜFFNER and many others, and the remarkable fact that the crystals in the salivary glands of hibernating *messeae* are with a few exceptions [1]) confined to the lateral lobes (DE BUCK and SWELLENGREBEL 1935), suggested to me that the secretion of the lateral acini might differ from that of the median one in its action on the blood. So I was led to a series of experiments in continuation of former observations on the salivary secretion of *Anopheles maculipennis* in the Netherlands (DE BUCK, SCHOUTE and SWELLENGREBEL 1932).

After a few preliminary experiments I gave up the technique of crushing the glands in saline under a coverslip. Though by this method, better than by teasing them out with needles, all the secretion is expelled from the cells, it necessitates the use of a fairly large quantity of saline to wash the coverslip. The technique I have adopted in the experiments recorded in this paper is very simple. The organ which is to be extracted is put in

[1]) In addition to the one exception described already I found one case more among 200 *messeae* with crystals in their glands. But whereas the cells of the lateral lobes in that case were crowded with bundles of large needles, the median lobes only contained the minute needles which require very close examination not to mistake them for an optical illusion as is often produced by the sticky secretion expelled from the glandular cells. For the rest in many other cases these minute needles are found in the lateral lobes too.

a drop of distilled water on a slide and allowed to dry. After drying the remainder is dissolved in a drop of saline. In applying this method I had the opportunity of observing a curious difference in the behaviour of the different acini of *A. maculipennis* when transferred to the distilled water. The secretion of the lateral acini escapes in the form of clear drops all over the surface of the distal portion of the acini, whereas the median lobe is seen to rupture, mostly in one spot only, the whole contents bursting forth from the opening in the form of a wrinkling worm-like mass. In the salivary glands of *A. plumbeus, Culex pipiens* and *Theobaldia annulata* this phenomenon cannot be observed.

1. *Agglutination of the red blood corpuscles.*

The salivary secretion of *A. maculipennis* contains a strong haemagglutinin. My experiments have shown that this is present in the median acinus only. The juice of the lateral acini causes no trace of agglutination. These experiments were performed with blood of man, monkey *(Macacus rhesus),* dog, goat, guinea-pig, rabbit, white mouse, chicken. The blood of all these animals yielded the same positive results [1]).

The haemagglutinin may be present in the median acinus in very great concentration. In many cases complete and immediate agglutination can be observed, when the secretion of the median acini of one mosquito is dissolved in 2 cc. saline, if the quantity of diluting saline is raised to 20 cc. a slight degree of agglutination may still be observed.

According to YORKE and MACFIE (1924) the haemagglutinin is destroyed by dessiccation, but it is my experience that the agglutinating power of the salivary secretion is not destroyed by preserving it for a long time in a dry state. A number of slides prepared in the usual way on February 7, each with the median lobes of one female *atroparvus,* were kept for over two months until April 20. On this date no loss of agglutinating property could be detected. Later on, however, on July 20 other slides prepared on February 7 and 21, yielded negative results. I was inclined to ascribe this to the circumstance that in summer the laboratory is not heated and consequently the humidity of the air might have been too high. This explanation is contradicted by the positive result of other slides, prepared on April 9 and kept for two months longer in the unheated room (until October 5). In this experiment, however, each slide carried the median lobes of three females (instead of one). Moreover the agglutination in some of them was not very strong.

Heating the secretion dried on slides at 99° C. for one hour does not destroy the haemagglutinin. Heating the solution of the dried secretion in saline (in a capillary glasstube with sealed ends) does not destroy the agglutinin if the heating is not carried over 40° C. for 15 minutes. If the

[1]) YORKE and MACFIE (1924) had negative results with monkey, guinea-pig and mouse; SHUTE (1935) with white mouse.

temperature is raised to 45° for 10 minutes or to 50° for 5 minutes the agglutinin is weakened. It is destroyed if the heating is continued at 50° for 15 minutes.

The salivary secretion of *A. plumbeus* and *Culex pipiens* does not possess any agglutinating property.

The salivary secretion of *Theobaldia annulata,* both of median and lateral lobes, in many cases causes a very light agglutination which can be made more distinct by using a greater number of glands. After heating the secretion in saline at 55° for 10 minutes there is no trace of agglutination even when the glands of ten females are used. This seems to indicate that the glands of *Th. annulata* contain a haemagglutinin which is of the same nature as that of *A. maculipennis,* but in a very small concentration. Accordingly the blood in the stomach, immediately or shortly after feeding, exhibits no agglutination.

2. *Delay of coagulation of the blood.*

In these experiments, which were performed with the blood of man and chicken, the solution of the dried secretion in one drop of saline was mixed with the same quantity of blood and the mixture drawn up into a capillary glasstube. The salivary secretion of *A. maculipennis* contains a strong anti-coagulin. My experiments have shown that this is present almost exclusively in the median acinus. The secretion of one single median lobe suffices in most cases to delay coagulation for hours, whereas the secretion of four or eight lateral lobes of one or two females never showed a trace of anti-coagulative action. But in many cases there was a marked delay in coagulation, if the number of females providing the lateral acini was raised to three or more.

In some cases with the lateral lobes of 3 females coagulation set in after 20 minutes, in one case with the lateral lobes of 8 females it did so after 3 hours. In many cases with 5 to 9 sets of lateral lobes coagulation set in within 30 minutes. In one case with the median acini of 10 females coagulation was prevented altogether, with the lateral acini of the same females coagulation set in after 30 minutes.

Still, when dissolving the secretion in a very minute drop of saline and mixing it with the same amount of blood, which requires the use of a capillary tube of narrow bore, even the secretion of the lateral acini of one single female may cause a delay in coagulation of as many as 30 minutes.

In most cases the anti-coagulin is present in the median lobe in a smaller concentration than the haemagglutinin. As a rule a distinct delay of coagulation is not observed with dilutions greater than 2 cc. of saline to the glands of one mosquito. It should be borne in mind, however, that it is much easier to verify the slightest amount of agglutination than a one minute's delay of coagulation.

In many experiments coagulation was prevented by the stomach

secretion. Whenever the stomach was split lengthwise and washed in saline, clotting took place normally. At first I was inclined to accept this as an evidence that the anti-coagulative action in these experiments was caused by salivary anti-coagulin, which by washing was removed from the stomach. However, the anti-coagulative property of the stomach secretion cannot be explained in this way for the following reasons. Firstly, after heating the dissolved stomach secretion at 80°, which does not destroy the salivary anti-coagulin (see further down), clotting took place within a normal time. Secondly, the anterior portion of the mid-gut did never cause any delay of coagulation, which proves that there is no overflow of the salivary glands to that part which is nearest to the salivary duct. I think the prevention of coagulation by the stomach secretion must be ascribed solely to the action of the digestive enzymes. By washing the stomach an excess of the enzymes is removed, so that coagulation may set in before the substances concerned in blood coagulation are impaired by digestion.

The anti-coagulative power of the salivary secretion is not weakened by prolonged drying. Dried secretion of the median lobes prepared on February 7 and April 9 and examined on July 20 and October 5 respectively showed no loss of anti-coagulative power.

Heating the dried secretion at 99° C. for one hour and boiling the dissolved secretion for 35 minutes do not destroy the anti-coagulin.

The salivary secretion of *A. plumbeus* contains an anti-coagulative substance which is not confined to the median acini.

The salivary secretion of *C. pipiens* (both the autogenous and anautogenous races) contains an anti-coagulin which is present in the lateral acini as well as in the median acini. In consequence of the small size of the glands it is necessary as a rule to use a greater number of mosquitoes in preparing the secretion. Still in many cases one set of glands or even one pair of the very minute median acini is sufficient to prevent coagulation for hours. In this respect the physiological condition of the glands of *C. pipiens* appears to vary within fairly wide limits. Hibernating Culex, newly caught in their winter-haunts, usually carry a weak anti-coagulin. By spending a week at room-temperature, on a diet of sugar and water, the anti-coagulative power of their salivary secretion is greatly increased. The minuteness of the median lobes may account for the fact that their anti-coagulative action is often much weaker than that of the lateral lobes.

The anti-coagulin is not destroyed by prolonged drying.

Heating the dried secretion at 99° C. for one hour does not destroy the anti-coagulin.

Heating the dissolved secretion at 45° for 30 minutes does not destroy the anti-coagulin. Heating it at 50° for 15 minutes weakens the anti-coagulin, heating it at 50° for 30 minutes destroys or weakens the anti-coagulin. Higher temperatures are invariably deleterious.

The salivary secretion of *Th. annulata* contains an anti-coagulin which is present in median and lateral acini, but in the former in greater concentrations than in the latter. It is not present in the basal portion of the lateral lobes. In many cases one set of median or lateral lobes is sufficient to prevent coagulation for hours.

The anti-coagulin is not destroyed by prolonged drying.

Heating the dried secretion at 99° C. for one hour and the dissolved secretion at 50° for 30 minutes does not destroy the anti-coagulin. Heating the dissolved secretion at 55° for 15 minutes destroys the anti-coagulin.

The salivary glands of the male *Th. annulata* do not contain any anti-coagulative substance.

3. *Acceleration of coagulation of the blood.*

The secretion of the stomach of *Th. annulata* contains a strong coagulin. When mixed with blood it has a marked accelerating action on coagulation. When mixed with blood which has previously been treated with salivary secretion of *Anopheles, Culex* or *Theobaldia,* it causes coagulation, either immediately or within a few minutes, the controls remaining unclotted for hours.

This coagulin is not present in the anterior portion of the midgut, nor in the stomach of the male.

The coagulin is not destroyed by prolonged drying.

Heating the dried secretion at 99° C. for one hour and the dissolved secretion at 40° for 30 minutes does not destroy the coagulin. Heating the dissolved secretion at 50° for 15 minutes destroys the coagulin.

A stomach coagulin is also present in *C. pipiens,* though in much smaller concentration than in *Theobaldia*, but not in *A. maculipennis.*

4. *Reaction of the skin.*

The presence of agglutinin and anti-coagulin in the median acini only of the salivary glands of *A. maculipennis* caused me to investigate whether the secretion of the lateral acini might be responsible for the irritating and wheal-producing action of the saliva.

HECHT (1929) came to the conclusion that it is really the saliva which has this effect on the skin, but that the same toxic substance is present, though in much smaller concentration, in the other organs of the mosquito. My own experiments have confirmed this. In these experiments the different organs, which I dissected out in saline, were inoculated intracutaneously, on the point of a sharp needle.

Inoculation of the salivary glands of *C. pipiens* instantly produced a large irritating wheal and extensive hyperaemia, inoculation of the oesophageal diverticulum had a slightly weaker effect, while inoculation of the stomach and the ovary produced but slight hyperaemia and itching.

Inoculation of the salivary glands and the diverticulum of *Th. annulata*

had about the same effect as that of the glands of *Culex*. Washing the diverticular sac made no difference, which seems to indicate that the toxic action of this organ is not to be explained by salivary juice having found its way to it. The fact that inoculation of the diverticulum of the male *Theobaldia* produced a toxic effect, only slightly inferior to that of the female, points in the same direction. In judging these results it is to be borne in mind that inoculation of the diverticulum is much easier than that of the glands. Inoculation of the stomach and ovary had the same effect as in *Culex*.

Inoculation of the lateral acini of the glands of *A. maculipennis*, in a person who is very susceptible to the bites of Anopheles and has an intense secondary reaction on the following days, produced a large wheal and short itching, replaced by a red spot the next day, which on the third day, on rubbing it, began to swell and itch. The median acini had a much slighter effect, there being a small wheal only immediately after inoculation and a small red spot on the next day, which on the third day had left no trace. Inoculation of the diverticulum produced a small wheal only which soon disappeared, there being no secondary effect on the next day.

Inoculation of these organs in a person who has only a short primary reaction to the bites of Anopheles, had a weak effect in the case of the lateral acini only. After a few minutes slight hyperaemia and in some cases a small wheal appeared, with or without a very slight itching.

These experiments point to the conclusion that in *A. maculipennis* the toxic substance is more concentrated indeed in the lateral acini, than in the median acini and the diverticulum.

5. *Summary.*

1. The haemagglutinin in the salivary secretion of *Anopheles maculipennis* is present in the median acinus only.

2. This agglutinin is not destroyed by prolonged drying, provided that the atmosphere it is kept in is absolutely dry, nor by dry heating at 99° C. But it is destroyed by wet heating at 50° C.

3. The salivary glands of *Theobaldia annulata* contain a weak haemagglutinin.

4. The anti-coagulin in the salivary secretion of *A. maculipennis* is present almost exclusively in the median acinus.

5. The anti-coagulin of *A. maculipennis, Culex pipiens, Th. annulata* is not destroyed by prolonged drying, nor by dry heating at 99° C.

6. The anti-coagulin of *A. maculipennis* is not destroyed by boiling, that of *C. pipiens* and *Th. annulata* is destroyed by wet heating at temperatures higher than 50° C.

7. The secretion of the posterior portion of the mid-gut of *C. pipiens* and *Th. annulata* contains a coagulin.

8. This coagulin is not destroyed by prolonged drying, nor by dry heating at 99° C. But it is destroyed by wet heating at 50° C.

9. The secretion of the lateral acini of the salivary glands of *A. maculipennis* seems to be responsible for the irritating and wheal-producing action of the saliva.

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Mathematics. — *On a certain class of conformal mappings.* By CORNELIS VISSER. (Communicated by Prof. J. G. VAN DER CORPUT).

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1. *Introduction.* Let D denote the domain $x>0$ in the plane of the complex variable $z=x+iy$. Let E be a simply connected domain, interior to D, bounded by a simple continuous curve Γ, and such that for arbitrary large positive p the angular domain $|y|<p(x-a)$ is part of E if only a is sufficiently great. Then there exists a function $w=f(z)$, regular in E, that maps E conformally on D in such a manner that $w\to\infty$, $arg\ w\to\alpha$ when $z\to\infty$, $arg\ z\to\alpha\left(-\frac{\pi}{2}<\alpha<\frac{\pi}{2}\right)$. It is well known, further, that $f(z)$ may be defined on the boundary Γ such that it becomes continuous in every boundary point.

It follows from a theorem due to WOLFF [1]) that there exists a real constant l $(0<l\leqq\infty)$ such that

$$\frac{w(z)}{z} \text{ and } w'(z)\to l$$

when $z\to\infty$ and $\left|\frac{y}{x}\right|$ remains bounded.

[1]) J. WOLFF, Sur une généralisation d'un théorème de Schwarz. Comptes rendus Paris **183**, 500—502 (1926).