of aether are added to the fluid. All the bilirubin passes into the mixture of ice-vinegar and aether, which separates entirely from the fluid underneath. If this yellow-coloured aether is pipetted and placed in an ice-safe in a loosely covered watch-glass, we likewise observe that crystals are formed.

The accompanying picture is a micro-photograph of bilirubin crystals which we obtained from the ascites-fluid of a heart-patient, and from normal human serum.

Attempts to produce bilirubin-crystals by the above-mentioned method from the intensely yellow-coloured serum of two icterus-patients, led to a remarkable experience. If namely we placed the chloroform-solution which, as appeared from various reactions, contained much bilirubin, in the ice-safe, for the purpose of a slow evaporation, the yellow colour at a certain moment when, owing to the evaporation of the solvent the concentration had reached a certain value, suddenly passed over into a green one, evidently by a change of the bilirubin into biliverdin. The same phenomenon occurred when we evaporated the chloroform-solution in vacuo. It must be distinctly understood that this occurred only with the solution obtained from the serum of patients suffering from obstructive jaundice. We cannot give an explanation of this phenomenon. Most likely the icterus-serum contains substances promoting the oxidation of bilirubin into biliverdin.

Botany. — "*Gummosis in the fruit of the Almond and the Peachalmond as a process of normal life.*" By Prof. M. W. BEIJERINCK.

(Communicated in the meeting of September 26, 1914).

It has hitherto been generally accepted that the formation of gum in the branches of the Amygdalaceae always is a process of pathological nature. I have found that this opinion is erroneous, and that gummosis occurs normally in the fruits of the Almond (*Amygdalus communis*) and the Peachalmond (*Amygdalus amygdalo-persica*) DUHAMEL DUMONCEAU. [1])

[1]) In some Dutch nurseries the peachalmond is simply called "Almondtree". The difference is in fact very slight as it consists only in the drying up of the almond fruit before the epicarp opens, and the position of the flowers in pairs, whereas the fruit of the peachalmond remains fleshy even at the dehiscence, and its flowers are mostly single. Between leaves, flowers and branches no constant differences are found.

GRENIER et GODRON (Flore de France T. 1, Pag. 512, 1848) call the peach-

Contrary to what might be expected the phenomenon is the more obvious as the trees are better fed and more vigorous. In specimens on sandy grounds it can only be observed with the microscope.

As gummosis is the effect of a wound stimulus, it is of importance that this process also takes place in the normal development of the healthy plant. The subject is moreover of practical interest. All the chief facts relating to gum formation can almost unchanged be applied to the production of gums in general, of gum resins, and of resins, among which are substances of great medical and technical value. As the study of the influence of parasitism has made it possible to produce gum, and no doubt many of the other substances mentioned, in a more rational way than has been done till now, a short review of the whole subject seems not superfluous.

Wound stimulus as cause of gummosis. Poisoning, and parasitism also causes of this stimulus.

Gummosis in the Amygdalaceae is a process of cytolysis, whereby young cells, freshly sprung from cambium or procambium, and sometimes also young parenchyma, are more or less completely dissolved and converted into canals or intercellular spaces, filled with gum. In dissolved parenchymatous tissues usually remains of not wholly disappeared cell walls are found; the gum of the phloem bundles is more homogeneous, but always the microsomes of the dissolved protoplasm are found. The nitrogen of the gum springs from the dissolved protoplasm.

Formerly we proved [1]) that by such different causes as poisoning, parasitism and mechanical wounding gummosis may be experimentally

almond *Amygdalus communis* var. *amygdalo-persica*. At present the name *Amygdalus persicoides* (KOCH, SERINGE, ZABEL) is also used, as in the Hortus of the University of Leiden. The opinion that it is a hybrid is not sufficiently founded. When grown from seed the tree seems constant (see MEIJER's Conversationslexikon, Articles "Mandel" Bd. 11, p. 853 and "Pfirsich" Bd. 13, p. 782, 1896) and identic with the "English almond", of which DARWIN reproduces a stone (Domestication, 2nd Ed., Vol. 1, p. 858, 1875). The fruit is fleshy and bursts open, the kernel is edible, not bitter. At Delft sowing experiments have been going on a long time already, but under unfavourable circumstances. The root cannot resist the winter temperature of the soil, hence, grafting on the plumtree is required.

[1]) M. W. BEIJERINCK et A. RANT. Excitation par traumatisme et parasitisme, et écoulement gommeux chez les Amygdalées. Archives Néerlandaises, Sér. 2, T. II, Pag. 184, 1905. — Centralblatt f. Bakteriologie, 2te Abt., Bd. 15, Pag. 366, 1905. — A. RANT: De Gummosis der Amygdalaceae. Dissertatie Amsterdam, BUSSY, 1906.

provoked in many Amygdalaceae, as almond, peachalmond, apricot, peach, plum, cherry, and bird's cherry.

But these three groups of causes may all be considered from one single point of view, by accepting that gummosis is always the effect of a wound stimulus, proceeding from the slowly dying cells, which are found as well in every wound, as at poisoning and parasitism. These dying cells may change into gum themselves, but besides, exert their influence on cambium tissues to distances of some centimeters. This distance-influence is the principal effect of the wound stimulus. But poisoning by sublimate or oxalic acid, introduced under the bark, can as well excite gummosis as an incision or a wound by burning or pricking. Neither the dead cells nor the poison are the active factors here; *the stimulus proceeds from the slowly extinguishing cells, so that gummosis is essentially a necrobiotic process.* Probably the dying cells, after the death of the protoplasm, give off an enzyme or enzyme-like substance, a lysine, fixed during active life, but, which being freed by necrobiosis and absorbed by the young division products of the cambium causes their cytolysis. This reminds of the cytolysines of the animal body, originating when foreign cells are introduced, which liquefy the corresponding cells, for example the haemolysines which dissolve the red blood-cells. Furthermore of the bacteriolysines and of cytase, the enzyme of cellulose.

If the hypothesis of the existence of a "gumlysine" is right, — and I think it is, — this substance must be of a very labile nature, for when bark wounds are infected with gum, quite free from germs of parasites, no more abundant gummosis is observed than at mechanical wounding only. But a difference, however slight, will certainly exist.

Gummosis produced by wound stimulus.

The influence of this cause is best studied in the following experiment.

A deep wound, penetrating into the cambium of a branch of almond or peach, commonly soon heals completely, but it may be that gum flows from the wound. This is the case when the trees are in sap, thus in February or March at temperatures above 20° C. and below 33° C. The experiment succeeds best with cut branches in the laboratory. When the wounds are made in the open air in that season no gummosis ensues, the temperature then being too low.[1] In

[1]) If the wounds are infected with *Coryneum*, an extremely copious gum production follows in spring, as the parasite then finds abundant food in the branches. There is, however, no season when wounds, infected with *Coryneum*, do not sooner or later yield gum.

summer the cambium of the still longitudinally growing part of young green branches may be caused to form gum by punctures or incisions, but these wounds heal quickly, except when "kept open" by *Coryneum* or other parasites.

As to thicker branches, wounded in spring, the microscope shows the following.

Around the wound a great number of gum canals are formed in the cambium, about parallel with the axis of the branch, some centimeters long, which become the thinner and shorter as they are more remote from the wound. The canals are separated by the medullary rays, which are with more difficulty converted into gum than the phloeoterma. All the gum canals together form a kind of network, whose meshes are filled by the medullary rays. The whole network has the shape of an ellipse, the "gum ellipse", the wound lies in the lower focus towards the base of the branch. The stimulus extends over the ellipse, evidently farthest in the direction of the branch, less far towards the base and sideways. So it may also be said that the wound stimulus extends farthest opposite to the "descending" current of nutrient matter, following the phloem bundles, or along with the "ascending" water-current, following the wood. Evidently the gum canals are more easily formed in the better fed cells above the wound than in those beneath it, where the nutrition must be worse. This is especially obvious in ringed branches. Wounds in the cambium, directly above the ring produce much more gum than those immediately below. [1])

Under ordinary circumstances the branches, after simple mechanical wounding, are soon completely healed, and if the cambium at the outside of the gum canals then again begins to produce normal secondary wood, the gum canals may later be found back in the wood itself. [2]) Evidently the healing takes place as soon as the stimulus ceases, and so it is not strange that when it continues by poisons or parasitism the gum production also continues.

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1) The nature of the power, by which the food transmitting, "descending" sap current moves through the phloem bundles, is not known. It is thus not impossible, that if the cause of gummosis is of a material nature, a lysine, moving through the tissues, it is able to run in opposition to the "descending" current. I think, however, that the extension of the stimulus does not go along the phloem but along the xylem bundles and the young wood, with the "ascending" sap.

2) I have never seen distinct gum canals in the secondary wood, but according to the descriptions they occur eventually.

Parasitism as cause of gummosis.
The connection between wounding and parasitism.

Wounds in peach branches treated with poisonous substances, such as sublimate, produce gum much longer and more copiously than the like wounds without sublimate. Other poisons have quite the same effect. Now it is clear that the direct influence of parasitism on the organism must be sought in the action of some poisonous substance. Hence it seems certain that what these three causes have in common, namely necrobiose, or the slowly dying of the cells surrounding the dead ones, is the base of gummosis, and that parasitism, where necrobiose lasts as it were endlessly, must be the most powerful instigator of the process.

That this simple view of the question has not yet taken root in science is proved by the most recent treatise on our subject by MIKOSCH,[1]) illustrated with beautiful anatomical figures. After the publication of Dr. A. RANT and myself of 1905, he described the relation of mechanical wounding to gummosis. But he did not think of poisoning experiments, nor has he any belief in the influence of parasitism on gum formation. WIESNER, in his recently published paper on gums in the new edition of his "Rohstoffe des Pflanzen reichs", is also of the same opinion as MIKOSCH.

For my object a short discussion of a few examples of parasitism will suffice.

The little caterpillar *Grapholitha weberiana* makes borings into the bark of plum and apricot, and if the outermost corklayer is removed by shaving it off, the butterfly finds so many fit places for deposing its eggs, that the larvae creep in by hundreds and make new borings from which later the gum flows out. These holes are coated with a layer of slowly dying cells, whence the stimulus extends, which produces the gum canals in the contiguous "cambium". By cambium I simply understand the not yet differentiated division products, "young wood" and young phloeoterma. The necrobiotic cells, clothing the continually extending holes in the bark, and the great numbers of new individuals of the caterpillars, make the gum production a chronical process.

To explain the formation of the enormous quantities of gum produced in this way, it seems only necessary to think of mechanical wounding and not of any special excretion from the animal. But it must be noted that the space, where the caterpillar lives during its

[1]) Untersuchungen über die Entstehung des Kirschgummi. Sitzungsber. d. Kais. Akad. d. Wiss. in Wien. Mathem. naturw. Klasse. Bd. 115, Abt. 1. Pag. 912, 1906.

growth, namely a vertical narrow canal in the innerbark, very near to the cambium, could not possibly be imitated artificially.

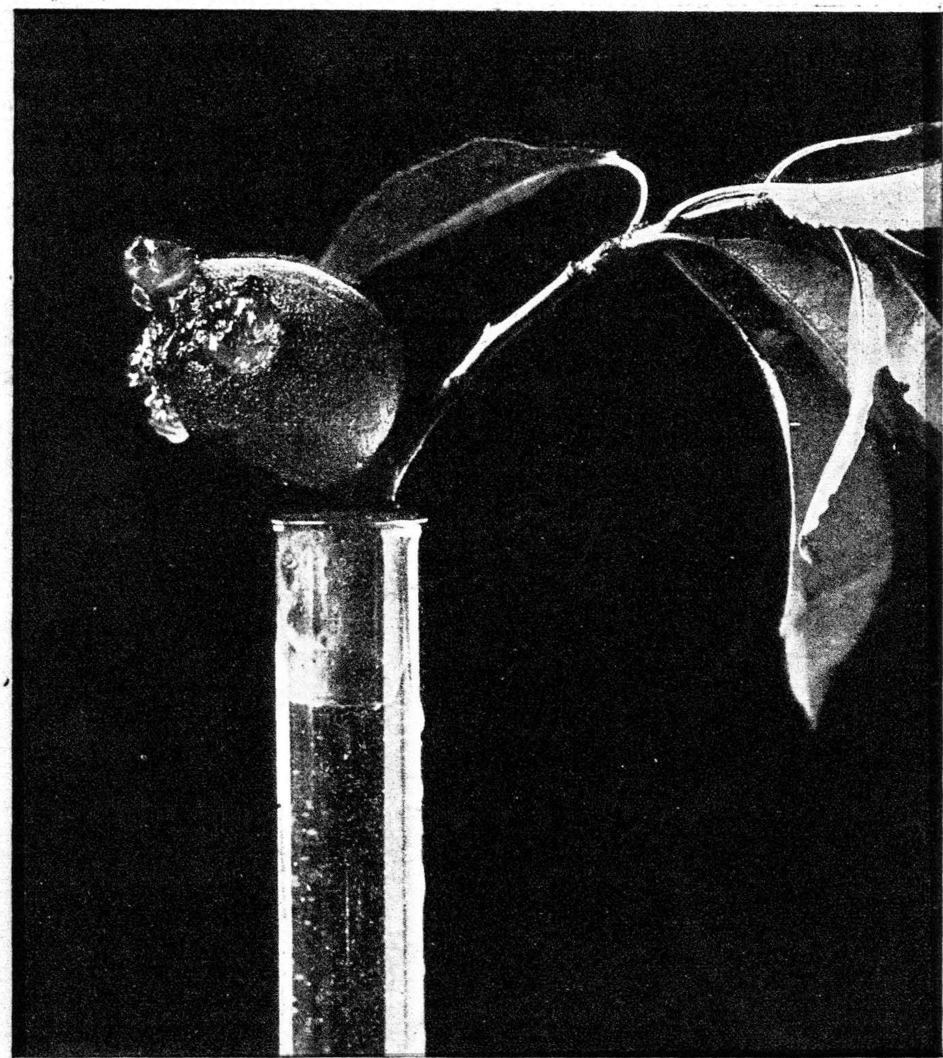

Fig. 1. (Natural size). Gum producing peachalmond in September, whose summit is cut off; the gum from the gum canals is after drying, swollen by moistening with cold water.

Much more common and interesting than the animal parasites are the gum producing Fungi of the Amygdalaceae, five of which are found in our country.[1]) The commonest and most vigorous is *Cor-*

[1]) *Coryneum beijerinckii* OUDEMANS, *Cytospora leucostoma* PERSOON, *Monilia cinerea* BONORDEN, *Monilia fructigena* BONORDEN and *Botrytis cinerea* PERSOON (see RANT, l. c. p. 88). German authors also mention bacteria as instigators of gummosis, I never found them.

yneum beijerinckii OUDEMANS (*Clasterosporium carpophilum* ADERH.).[1])

Pure cultures of *Coryneum* in bark wounds of almond, peachalmond, peach, cherry, plum, bird's cherry, sloe, virginian plum, develop with remarkable quickness and soon make the bark die off, evidently in consequence of the secretion of a poison. Around the dead cells the necrobiotic are found from which the stimulus issues, which, penetrating into the cambium in the usual way, forms gum canals in the young wood. Many mycelial threads of the parasite itself are then cytolised and converted into gum. I think this fact remarkable and a strong argument for the material nature of the stimulus.

Undamaged branches are with difficulty infected by the parasite, but it is easy, even by very slight wounds and artificial infection, if only the wounds be numerous, to obtain great quantities of gum. This circumstance explains why nursery men dread wounds in the trunks and branches of stone-fruit trees.

In the green shoots, especially of the peach, the formation of anthocyan is observed in the enfeebled tissue around the wounds infected with *Coryneum* when exposed to sunlight. [2])

The supposition that secretion products of the parasitic caterpillar or the Fungus could be the direct cause of the stimulus, is contrary to the positively existing relation between mechanical wounding and gummosis.

Gum canals in the fruitflesh of almond and peachalmond.

To the preceding facts, long since stated, I wish to add the following.

Already in my first paper of 1883 I called attention to the circum-

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1) BEIJERINCK, Onderzoekingen over de besmettelijkheid der gomziekte bij planten. Versl. d. Akad. v. Wetensch. Amsterdam, 1883. — Contagiosité de la maladie de gomme chez les plantes. Archives Néerlandaises, 1é Sér., T. 19, Pag. 1, 1886. — C. A. J. A. OUDEMANS, Hedwigia, 1883, N⁰. 8. — SACCARDO, Sylloge Fungorum, Vol. 3, Pag. 774; 1884. — ADERHOLD, Ueber *Clasterosporium carpophilum* (LÉV.) ADERH. und dessen Beziehung zum Gummifluss des Steinobstes. Arbeiten der Biolog. Abt. am Gesundheitsamte zu Berlin. Bd. 2, Pag. 515, 1902. ADERHOLD has experimented with pure cultures of *Coryneum*, which I had made and sent him. He himself has not executed any isolations of gum parasites. His determination as *Clasterosporium amygdalearum* (LÉV.) is thus founded on the imperfect descriptions from the older mycological literature, in which OUDEMANS was no doubt better at home than he. Like LINDAU I reckon *Clasterosporium* to another family than *Coryneum*.

2) The apperance of anthocyan in the light is commonly a token of diminished vitality and often a consequence of necrobiose in the adjoining cells. Hence, wounds, poisons and parasitism cause anthocyan production in the most different plants.

stance, that in the fruit-flesh of the peachalmond, and as I may add now, also in that of the almond itself, there is a system of gum canals, precisely corresponding to that of the vascular bundles. Of these the phloem bundles are converted into gum canals by cytolysis, either entirely or with the exception of the outer protophloem; the gum canal (*gp* Fig. 2 and 3) thus, is always immediately contiguous to the woody bundle *xl*.

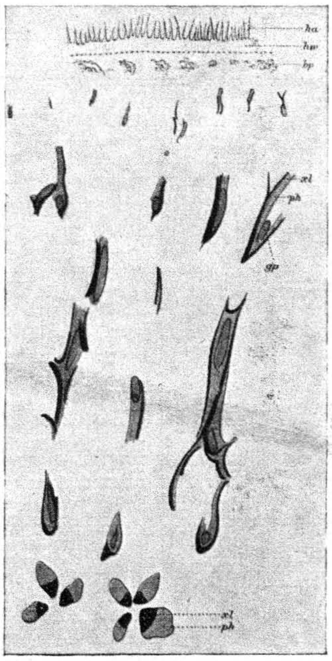

Fig. 2 (3). Gum canals in the transverse section of the fruit-flesh of a peachalmond: *ha* hairs on epidermis; *hw* dermoidal tissue; *bp* chlorophyll-parenchyma; *xl* xylem bundles; *ph* phloem bundles; *gp* gum canals sprung from phloem bundles.

Fig. 2 and 3 are reproductions from my above mentioned treatises of 1883 and 1886.

The presence of gum in the canals of the fruit is easily shown. In August or September the summit of a peachalmond fruit is cut off and the fruit, or the branch with the fruit, is placed in water. After some moments all over the section droplets of gum are seen evidently issuing from the vascular bundles. As these bundles are distributed through the fruit-flesh, running longitudinally and transversely, and are partly reticulated, the number of droplets is very great and they are of different size. In particular near the stone they are big. If in August the gum is allowed to flow out in cold water it dissolves completely or nearly so. In September the dissolving is no more complete. By drying the gum, its solubility in cold water gets almost lost, but it continues in hot water.

From lateral incisions also much gum flows out. In Fig. 1 the drops are represented after drying, followed by swelling up in cold water.

Although this gum does not only consist of dissolved wall material but also of cell contents, the microscope can only detect fine granules, evidently corresponding to the microsomes of the protoplasm, which are not dissolved during the cytolysis I could not find back the cell nuclei in the gum, but in the cells of the not yet cytolised phloem bundles, they are neither perceptible. As under normal circumstances the gum does not flow out, its volume must be about as great as that of the phloem bundles which are cytolised. It is, however, certain that the capability of the gum to swell up by imbibition is much greater than that of the cell-tissue which gave rise to its formation. It seems thus certain that imbibition with

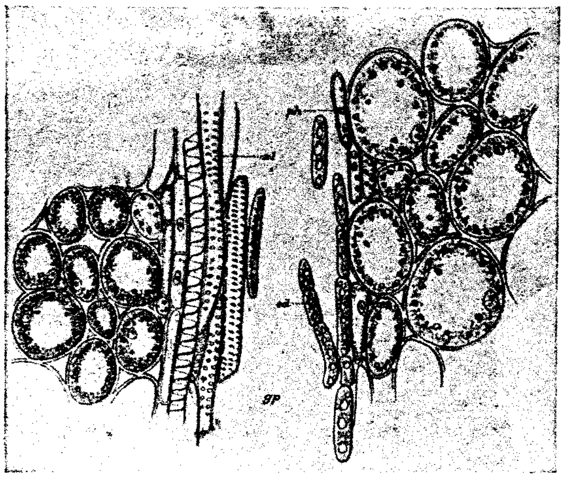

Fig. 3 (360). Gum canal with surrounding; *gp* gum; *xl* xylum bundles, unchanged; *ph* non-dissolved cells of the phloem bundles; *cd* thread-shaped cells in a gum canal, originating from the phloem bundles.

sufficient access of water must lead to a perceptible pressure and also some thickening of the fruit-wall. This must promote the opening of the fruit as well as the remarkable detaching of the stone, although the required mechanical power for these processes must, no doubt, chiefly be the tension of the tissue of the parenchyma of the fruit-wall existing independently of the gummosis. Finally the stone is found quite loose within the fleshy shell, which mostly opens like a bivalvate mollusk, but sometimes shows three or four fractures. The vascular bundles, which pass from the fruit-flesh into the stone, are thereby torn off clear from the stone. At the base the separation seems provided for by an intercepting-layer, as at the fall of leaves.

The portion of the phloem bundles within the stone of the peachalmond is never converted into gum ; in the almond itself such gum is found in rare cases inside the shell.

Wound gum in the fruit-wall as a consequence of mechanical stress of the tissue. Gumming almonds.

In many cases real wound gum is found in the fruits of the almond and the peachalmond, not proceeding from the gum canals but from fractures in the parenchyma of the fruit-flesh. Its origin must undoubtedly be sought in the tension or stress of the tissue, which causes the opening of the fruit. An additional circumstance, however, is required, namely a loss of vital strength, by which the regenerative power of the tissue that coats the fracture is annihilated. The therefrom resulting incapability of regeneration is associated with the ripening of the fruit in a way not yet explained and should rather be attributed to superfluous than to poor nutrition. Parasitism is wholly absent in the production of wound gum from the parenchyma of the fruit.

The fracture is mostly at the side where the two edges of the carpels are grown together and the fruit later opens. Not seldom in this case is wound gum seen to flow spontaneously from the base of the fruit along the short peduncle. In other cases the wound is at the side of the middle nerve of the carpel. Always the edges of the fracture are coated with cells in a condition of necrobiose, which is evident by their quickly colouring brown at the air, which normal living cells do not. These necrobiotic cells and the adjoining tissue produce gum. With the microscope not quite dissolved cell-walls may be found in the gum, showing that the cells were about full-grown when the process began.

In common almonds gum is sometimes found *within the hard shell,* [1]) and eventually part of the kernel itself is then also changed into real wound gum with still recognisable remains of the cell-wall. In such almonds the phloem of the vascular bundles, which run through the stone to the funiculus, is always changed into a gum canal, so that the gum can reach the surface of the young seed.

If we suppose that gummosis originates by the action of a cytolysine, it seems very well possible, that the lysine which has flowed inward together with the "canal gum", is able to attack the developing

[1]) The small quantity of gum found, especially in "hard almonds", at the surface of the shell, proceeds from the gum canals of the fruit-flesh. The sugar layer which covers the shell of the "soft" species is dextrose.

seed and is yet too labile to be demonstrated by infection of bark wounds with gum. Experiments in this direction may perhaps be effected with the peachalmond.

Wound stimulus as factor of development.

Formerly I thought that the presence of gum canals in the fruits was accidental and should be explained by parasitism, although I could not find any parasites.

In later years, with better knowledge, I again examined the gum canals in the peachalmond and their surroundings repeatedly. Never did I find a fruit without them, but they were not equally developed in different trees from different gardens. In specimens of sandy grounds they can sometimes only be found with the microscope. Neither microscopically nor by experiments has it been possible to detect gum parasites. This makes it quite certain that in the formation of gum canals parasitism is excluded. [1])

The great ease wherewith mechanical tension causes wounds in the fruit-flesh of the peachalmond, gives rise to the supposition, that the normal gum canals may be the product of some hidden wound stimulus.

If this supposition is true, we cannot think of wounding in the common sense of the word. When the flowers fall off, a ring-shaped wound forms around the base of the young fruit, but this is a normal process, taking place in an intercepting layer and soon followed by complete healing. In the flowers of peach, plum, apricot, cherry, we observe the same without any formation of gum canals in the fruit-flesh. Moreover, although the peculiar structure of the layer between the woody peduncle and the stone, along which the ripe fruit detaches, reminds of rent tissue, no gum is formed at that spot and the layer also exists in the other stone-fruits, where no gum canals occur.

So long as nothing else has been proved it must therefore be accepted that in the phloem bundles of the fruit of the peachalmond, where cytolysis takes place, the same factor of development is active as that, which gives rise to the pathological gum canals in the cambium of the branches. This leads to the conclusion, that the wound stimulus belongs to the normal factors of development of this fruit, although nothing is seen of external wounds. When considering, that the phloem bundles are built up of extremely thin and soft-walled cells,

[1]) The supposition, sometimes met with in literature that the gum of the Amygdalaceae should consist of bacterial slime is quite erroneous. That parasitic bacteria eventually occur as gum parasites, as is stated by some authors, I do not think impossible, although till now I only found caterpillars and Fungi as active agents.

it is conceivable, that by great tension of the tissue in the surrounding parenchyma, they undergo strain and pressure causing mechanical rupture and necrobiose, centre and prey of the wound stimulus being the phloem bundles themselves.

This conception is in accordance with the fact that the gum canals are broad in the fruits of well-fed trees on rich grounds, which have a hard and solid flesh, wherein stress and strain are certainly very great. Only here and there remains of the protophloem along the gum canals are still to be found in such fruits. But in the softer fruits of sandy soils, along the much narrower gum canals not only the protophloem is still present, but also stripes of the secondary phloem.

Summarising we come to the following conclusions.

Mechanical wounds in growing tissues of Amygdalaceae will sometimes heal directly, sometimes after previous gummosis.

The chief tissue, which is transformed into gum is the young secondary wood newly sprung from the cambium and not yet differentiated. By the wound stimulus a network of gum canals is formed around the wound. In thick branches, with a bark wound, this network has an elliptical circumference, the wound being in the lower focus of the ellipse.

If the stimulus is removed by the cure of the wound, the cambium again continues to produce normal secondary wood, so that afterwards the gum canals may be found in the wood itself.

If the stimulus continues the gum formation also becomes lasting.

The stimulus issues from the cells that die slowly by wounding, poisoning or parasitism. Probably a cytolysine flows from these cells into the young wood or the procambium; these bind the lysine and liquefy to gum. Hence, gummosis is caused by necrobiose.

Young medullary rays and phloembundles are with more difficulty converted into gum than the young secondary wood. But in the fruit-flesh of the almond and the peachalmond it is the phloem which changes into gum. The protophloem of the bundles often remains unchanged.

Although gummosis in these fruits belongs to their normal development, a wound stimulus is nevertheless active. This stimulus springs from the strong tension in the parenchyma of the fruit-wall, which gives rise to tearing, necrobiose and gum formation in the delicate tissue of the phloem bundles. Consequently the wound stimulus is here a normal factor of development.

It might also be said that the almond and the peachalmond are pathological species, but thereby nothing would be explained.