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A PECULIAR NUCLEAR SAFRANIN REACTION AND ITS RELATION TO THE CARCI- NOMA COCCIDIA QUESTION.

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BY A. P. OHLMACHER, M.D.,

PROFESSOR OF EMBRYOLOGY AND BIOLOGY, COLLEGE OF PHYSICIANS
AND SURGEONS, CHICAGO.

The employment of a large number of chemical reagents is one of the unfortunate necessities of modern microtomy. No one can become familiar with the long list of substances employed in microscopical research, nor with the bewildering combinations of fixing agents and staining agents, without realizing the possibility of many of these substances being used without a definite knowledge of their effects. The history of microscopic anatomy is full of instances, in which the results of some pioneer investigator have been attacked, on the ground that the results which he obtained were the effects of the reagents employed in the study. In fact, it is even now not uncommon to have the question of the reagent effect brought up, when intricate microscopical studies are being prosecuted. Without doubt, the suspicion that many of the marvellous findings of the cytologists are the products of unknown micro-chemical changes, is still entertained by many individuals.

The controversy between Henle,¹ Flemming,² and others, concerning the nuclear fragmentation observed in leucocytes and pus corpuscles, is a notable instance of the skepticism relating to the interpretation of microscopical findings. Henle maintained that the multi-nuclear condition of leucocytes and pus corpuscles was the result of the acetic acid, or other reagent, used in studying these bodies. This view was strongly opposed by Flemming, who observed multi-nuclear leucocytes in various situations in living objects, and who further noted that no change was produced in these cells by killing them with acetic acid, save that the nuclear structure became more apparent.

Every histologist will remember the discussion which arose when the discovery of the reticulated condition of the protoplasm of the cell and nucleus was announced; a considerable number of investigators arraying themselves on the side which maintained that the reticulum was either a faulty optical interpretation, or was the product of the changes induced by the chemical substances employed in the technique. But Flemming and his followers apparently put the subject at rest, by not only demonstrating the nuclear and cytoplasmic reticulum, but

also by following the processes of direct and indirect nuclear division in *living tissue*; these observations being made by the aid of the most perfect optical appliances.

While the employment of reagents is a prime necessity of microscopical technique, it is nevertheless a fact, that such reagents produce a variety of compounds with each other and with the substances of the tissue, the exact natures of which are either unknown, or but imperfectly understood. It follows then, that until our knowledge of ultimate micro-chemical cellular and nuclear reactions be complete, we must regard with a reasonable skepticism, many of the startling microtomical novelties of the day.

The open questions in this connection are many. What is the exact nature of that part of the so-called chromatin of the nucleus, which retains certain anilin stains when employed by the "indirect method?"³ What is the effect of the fixing medium on this chromatin? What is produced by the combination of the nuclear chromatin and fixing agent, which allows a certain dye to stain precisely, and another one to fail? Many queries of this character confront the microtometist at every turn, and impress him with the profound character of the many unsolved biological problems. It cannot be said, however, that the absorbing subject of the micro-chemistry of staining agents, particularly the anilins, has been entirely neglected; for the work of Ehrlich⁴ and his students on the reactions of leucocytes, and "mastzellen," indicate the tendency to investigation in this direction.

In this connection also, may be mentioned the work of Auerbach,⁵ Watasé,⁶ and others, on the two chromatin substances which are distinguished by their micro-chemical and staining reactions. These authorities go so far as to indicate the *sexuality* of the two nuclear substances; the erythrophile, being the preëminently male constituent, and the cyanophile, essentially female. Manifestly, such work opens a wide field for research, the bearings of which must be felt in every department of biological or pathological investigation.

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Among the many staining substances employed in the new microtomy as inaugurated by Böttcher and Hermann and improved by Flemming, one, safranin, has come into very extensive use. When employed after the "indirect" or Flemming method, safranin gives a precise chromatin stain, which compares in color and durability with carmine. The solutions of safranin in common use are two, viz., the alcoholic

³ A. B. Lee. The Microtometist's Vade Mecum. Second edition, London, 1890, p. 55.

⁴ Farbenanalytische Untersuchung zur Histologie und Klinik des Blutes. Gessamm. Mittheil. v. P. Ehrlich, I, 1891.

⁵ Ueber einen sexuellen Gegensatz in der chromophilie der Keimsubstanzen neiderer Wirbeltheire. Von Prof. Dr. L. Auerbach. Aus Sitzungsberichte der königl. preussischen Wissenschaften zu Berlin.

⁶ On The Phenomena of Sex-differentiation. S. Watasé. Jour. Morphology, VI, No. 3, 1892.

¹ Henle. Zur Entwicklung der Krystallinse und zur Theilung des Zellkerns. Arch. f. Mik. Anat., Band 20, p. 413, 1881.

² Flemming. Zellschubstanz, Kern und Zelltheilung, Leipzig, 1882, p. 348.

(Pfitzner's and Flemming's), and the anilin-water (Babes, Zwaardemaker). It is very necessary that the safranin employed be of the right quality and in this respect the products of Grüber meet all requirements: his "safranin spirituslöslich" being adapted for the alcoholic solutions, and the "safranin wasserlöslich" for the anilin water solutions. These staining solutions can only be employed with sections of tissue; and it is further true, that safranin only gives its best result in sections of tissues which have been fixed in a chromic acid mixture. The practice of safranin staining consists in allowing the section to attain an over-stained condition in the coloring solution, and then washing out and fixing the stain, then clearing the dehydrated section, and mounting it in balsam. A variety of agents have been employed in the washing and fixing of the safranin, as acidulated alcohol (Flemming⁷), strong picric acid alcohol (Podwyszoński⁸), iodine after Gram's method (Prenant⁹), and chromic alcohol (Martinotti and Resegotti¹⁰).

The action of the hydrochloric acidulated alcohol, or plain alcohol, when employed in washing out, seems to be merely a solvent one. The excess of stain dissolves in the spirit, and the remainder is held by the nuclei, which apparently have the greatest affinity for the color. With chromic acid, or iodine, however, the effect seems different, as suggested by Lee,¹¹ who considers it probable, that these agents have a sort of "mordant" action on the nuclear chromatin, inducing in it a marked affinity for the staining material. In this connection it is important to direct attention to the fact, that chromic acid, picric acid and iodine, all possess the property of precipitating safranin from its solutions. The experiment may be tried by adding a drop of the washing and fixing mixture (chromic acid solution, picric alcohol or iodine solution) to a drop of the safranin solution on a glass slide; here the precipitation may be carefully observed by the aid of the microscope. In the case of the chromic acid solution, an amorphous yellowish precipitate is instantly produced when a drop is brought into contact with the safranin mixture. With an alcoholic iodine solution, *a mass of deep red, needle-shaped crystals is produced immediately*. When picric acid alcohol, and anilin water safranin solution are mixed, the precipitation is much more tardy; but sooner or later, a crystalline deposit may be observed *which resembles very closely that produced by iodine and safranin*. All of these deposits are soluble in alcohol.

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In a modified method recently proposed, Heidenhain¹² advises the employment of tincture of iodine in treating the sections of tissue fixed in corrosive sublimate, previous to staining, with the object of removing the crystals of sublimate which often remain in the tissue. This process consists in treating sections of paraffine imbedded tissue, which have been fixed on the slide, and previously treated by xylol and alcohol, with strong tincture of iodine. After the iodine has acted ten or fifteen minutes, the section is washed in changed alcohol, and is then ready for any kind of staining.

While engaged in the preparation of some frog's tissue after Heidenhain's method, which was to be stained in safranin, I made the mistake of placing the section in the staining solution (Pfitzner's alcoholic safranin mixture) before it had been treated with the tincture of iodine. It occurred to me to try the tincture of iodine *after* the safranin, in the hope of still being able to remove the sublimate crystals.

The first section on which the procedure was tried was from the heart of the frog, which had been stained for twelve hours in the safranin. The iodine tincture was applied to the section immediately on its removal from the staining solution, and was allowed to act for two or three minutes. A brief washing with 95 per cent. alcohol followed, by means of which the excess of stain was removed and the section dehydrated. The object was cleared with clove oil, and mounted in xylol balsam.

On examining this section of the frog's heart, microscopically, I was struck by the remarkable appearance presented by the tissue. Under the low power (Leitz objective 3), the appearance of the section at first sight resembled that of precise safranin staining when carried on after usual methods; *i.e.*, the nuclei were prominent by their deep stain, while the ground substance appeared nearly unstained. More careful observation even by the low power, demonstrated a peculiar granular appearance in the nuclei, as something entirely unusual. On employing a higher power (Leitz objective 8), the nature of the granular appearance of the nuclei became more apparent. The nuclei of the heart muscle were exceedingly prominent, because of the presence in their interior, of irregularly arranged, deep red stained masses, quite distinct from the usual chromatin framework of such nuclei. At first superficial examination it seemed as if all the nuclei of the heart muscle were undergoing the process of karyokinesis, particularly, as some of the red stained masses assumed attitudes not unlike certain chromatic figures of indirect nuclear division. This idea was dismissed, however, on closer observation; for aside from the many usual shapes presented by these intra-nuclear bodies, it was noticed that the usual stages of karyokinesis were not reproduced, and that the nuclear membrane was always intact. The idea of the bodies being bacteria presented itself, but was at once abandoned, because of their irregularity in size and shape, and because of their constant presence within the nucleus.

A more prolonged study, and particularly with the aid of the twelfth inch oil immersion lens, brought out the characteristic features of these safraninophile masses. Their prominence was due to the intensity of their deep red color which allowed them to stand in relief, even when strong substage illumination was employed, as is the case with well stained bacteria in tissue, and with nuclear figures. The shape of these bodies exhibited all variations from the nearly spherical, to the rod-like; and the bodies were sometimes single and again thrown together in masses. The rod or stick-like forms predominated, and these varied greatly in their dimensions; and some exhibited blunt round ends, while others were pointed, or "lance-like." Undoubtedly, the variety of shapes was in part due to the relation of the bodies to the plane of the optical section. A little ingenuity in comparison would have made it easily possible to describe

⁷ Zeit. fur. Wiss. Mik., I, 3, 1884, p. 350.

⁸ Beitr. zur path. Anat., I, 1886.

⁹ Int. Monatschrift fur Anat., IV, 1887, p. 358.

¹⁰ Zeit. fur. wiss. Mik., IV, 3, 1887, p. 328.

¹¹ Loc. cit. p. 58.

¹² Ueber Kern und Protoplasma. Festschr., Albert von Koelliker, Leipzig, 1892.

among these bodies such forms as "lance-like," "whetstone-like," "sickle-like," "boat-like," and "button-like." The number of these formations in a nucleus, varied from half a dozen, to twenty or more; some nuclei showing but a few, others being entirely filled with them. The size varied from minute granules, to long masses which equaled and sometimes exceeded in length, the diameter of the nucleus. The color of the formations, as indicated above, was a homogenous deep reddish brown. Occasionally a mass of rod-like forms gave a yellowish or brownish reflex with strong illumination.

Figure 1 gives an idea of the variety in shape, size, and arrangement of the forms in question, and represents fairly well the deep color and its contrast with the pinkish hue of the nucleus proper.

More extensive study of the section with the high powers, brought out several interesting facts. In many muscle nuclei containing the safraninophile objects, the ordinary chromatin framework with its nucleoli could be detected by its faint pinkish color. Many nuclei of the muscular tissue were also noted, in which the pink chromatin framework was visible, but which contained none of the deep red bodies in question.

In the red blood corpuscles of this preparation, the peculiar formations occurred in some of the nuclei, but here the uniformity observed in the muscle nuclei was absent; and it was further observed that the safraninophile bodies when present, were much smaller than in the muscle. In fact, it may be here remarked, that the described formations bore a very constant relation to the size of the nucleus in which they were found. Often the nuclei of the red blood cells appeared a homogeneous deep red color; again, they appeared entirely filled with fine red granules; and finally many nuclei could be seen in which the deep red bodies appeared distinctly visible on a pinkish background. A pink chromatin network could occasionally be seen in the nuclei of the blood corpuscles. The three red corpuscles in Fig. 2, show the variations in the nuclear contents.

The leucocytes in the sections from the frog's heart prepared by the safranin-iodine method, usually exhibited a faint pink nuclear network, it being only exceptional to find nuclei containing the deep red safranin bodies.

A number of sections from the same piece of tissue were stained in the above described manner, and they uniformly showed these strange nuclear contents. Several control sections were cut from the same piece of tissue, stained in the same safranin solution, but washed in alcohol acidulated with hydrochloric acid (1 to 1000); and in these sections the bodies were never found, but the nuclei only presented the ordinary chromatin pictures.

As these remarkable appearances were noted, a number of queries arose as to the nature and significance of the strange intra-nuclear bodies. Were these striking forms a normal structure of the nucleus brought out by this peculiar staining combination? Were they artificial products of some combination of the reagents employed? If so, were they produced by a combination of the sublimate and iodine and colored by the safranin; or were they the result of the iodine and safranin reaction? To answer these questions, I proceeded to make a number of experiments on sections from a variety of animal tissues prepared with various fixing and staining

combinations. The normal tissues studied, were from the salamander, frog, snake, turtle, cat, dog, sheep, pig, ox, and man. The pathological tissue was mostly from carcinomata and sarcomata. The objects from these sources had been fixed in Flemming's solution, Hermann's solution, absolute alcohol, and corrosive sublimate (Heidenhain's solution). In all cases in which the safranin-iodine stain was employed, control sections were stained with safranin, gentian violet, or other stain, after the common methods.

As a summary of these experiments, I may say that it was possible to produce the characteristic safranin bodies in all of the tissue, no matter what its source, or in what medium it had been fixed. That is to say, the safranin-iodine staining combination developed the deep red formations in sections of tissue fixed in Flemming's solution, Hermann's solution, and absolute alcohol, the same as in sublimate fixed tissue. The only difference to be noticed in the effects produced by the fixing agents, was that the ground stain in sections of tissue from an osmic acid fixing solution, was of a more brownish hue, in contra-distinction to the pink ground color of the sublimate sections. This was particularly true of tissue which had remained long in the osmic fixing mixture.

While it was always possible to demonstrate the deep red bodies in some portion of a section, none of my subsequent preparations gave the remarkably uniform appearance of the nuclei of the heart muscle of the frog. Many times the nuclei of one portion of a section were either unstained or showed the normal chromatin framework, while the nuclei of another portion would contain the safraninophile masses. It was particularly noted that the nuclei about the borders of a section seemed to retain the safranin bodies most persistently. In some sections also, dark brown spots, visible to the unaided eye occurred, and on examining these spots microscopically, they were found to be composed of a dark red mass of safranin bodies which resembled very closely those found in the nuclei. Again it was noted, that the safranin bodies were not always confined to the nuclei, even in sections showing none of the large masses; for in many preparations they occurred in the cell protoplasm. Concerning this diversity in the amount and situation of the formations, it was found that it depended upon the duration of the washing with alcohol which followed the staining; a short washing leaving many of the bodies, and a more prolonged washing making them less numerous, and more precisely confined to the nucleus. As in ordinary anilin staining, a stage could be reached with prolonged washing, in which all stain could be removed from the section.

As stated above, the size of the safraninophile bodies varied with the size of the nucleus; and in the same degree the shape of the individual forms seemed to vary, so that, instead of the preponderance of the rod or stick-like forms of the frog's heart muscle, an indescribable number of shapes often presented themselves.

With regard to the behavior of the various tissue elements, besides the fact already mentioned, that the peculiar condition could be produced in the nuclei of all the tissue studied, I will only direct especial attention to the reaction of the non-nucleated red blood corpuscles of mammalian tissue. In normal tissue fixed in Flemming's solution, and

stained by the safranin-iodine combination, the red blood corpuscles usually assumed a uniform brownish tint, and the *safraninophile bodies were often found in their interior*. The significance of the observation will appear later.

Several experiments with various solutions were made, and it was found that the peculiar reaction could be produced in sections stained in Babe's anilin-water safranin, as well as with the alcoholic solution. The most perfect result was obtained when strong (U. S. P.) tincture of iodine was allowed to act a minute or two. Weaker solutions of iodine in alcohol did not act so perfectly, though the safranin bodies were still formed if the iodine was permitted to act longer. In the few experiments I made, it was impossible to produce the characteristic appearance when safranin stained sections were treated with an aqueous solution of iodine and iodide of potassium.

My conclusion as to the nature of these safraninophile bodies is that they are *artificial crystalline products, the result of a safranin and iodine reaction*. That their production is in no way due to sublimate fixation, as at first surmised, is clear from their occurrence in tissue fixed in other media. I believe them to be of a crystalline nature, not only because of their resemblance to crystals when examined with high powers, but also from the fact that crystals of similar appearance may be produced by mixing safranin solution and tincture of iodine on a slide, as previously mentioned. The fact that these crystals are soluble in alcohol would account for the varying appearance presented with the varying duration of the washing process, following the staining.

The remarkable affinity of these crystalline safranin bodies for the nuclei, seems to me due to the fact, that the nuclear chromatin naturally attracts the stain most strongly, and retains it most persistently; and it is in the nucleus, therefore, that the safranin-iodine reaction is most intense, the production of crystals most pronounced; and the nucleus is that portion of the tissue from which the crystals would be last dissolved in the washing out process.

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At the time in which I had made the observations on the safranin-iodine crystals, I was deeply interested in the investigations relating to the etiology of carcinoma, and it was at this time that the contribution of Podwyssozki and Sawtschenko¹³ came to my notice.

This work is of much importance in connection with the carcinoma organism question, not only on account of the high scientific reputation of the principal author, but also because of the very positive manner in which these investigators argue for the presence of coccidia in a variety of carcinomata examined by them.

After reviewing the literature of the subject, Podwyssozki proceeds to describe his own investigations, and claims to have found certain intracellular bodies in twenty cases of carcinoma examined by him, which he can interpret only as true sporozoa.

The technique which gave the best results to these authors, consisted in fixing the tissue in Flemming's solution, staining in anilin-water safranin, and washing in *picric acid alcohol*.

The bodies described as coccidia were round or

oval forms, of varying size, usually of a brownish tint, and containing in their interior safraninophile "nuclear" substance. It is upon the presence of the safraninophile substance in these bodies that the authors lay the greatest stress, and in the varying relations and situations of these deep red particles, the writers interpret the different developmental stages of the coccidia. Of especial importance is the presence of "sickle-formed" and "spindle-formed" red masses, in the bodies which they designate coccidia; and bodies filled with these "sickle-formed embryos" they consider as ripe individuals especially diagnostic of coccidia or sporozoa. Certain masses or conglomerations of the round or oval bodies with safranin "nuclear" substance, and usually found in the lymph spaces of the carcinoma, are looked upon as spore cysts. Aside from the method of propagation by spores, a form of direct division is suggested, in which a mass of "nuclear substance" is found at each pole of a coccidium.

These investigators remark that the only tissue element which assumes a brownish tint similar to the so-called sporozoa, in carcinoma tissue fixed in Flemming's solution and stained by the safranin-picric acid method, is the red blood corpuscle; but the feature which distinguishes their coccidia-like cells from the red blood cells, is the *presence of the safraninophile substance in the coccidia, and its absence in the blood corpuscle*.

In a paper published some time previous to that of Podwyssozki and Sawtschenko, Stroebe¹⁴ examined some fifty tumors, mostly carcinomata and sarcomata. He fixed his tissue in Flemming's solution, and stained the sections in safranin, then washed them in alcohol acidulated with hydrochloric acid, and finally with *alcohol to which picric acid was added*. In studying these preparations he noticed, aside from the usual chromatin network, homogeneous, round or oval bodies, containing deep red formations of a "lancet" form, "whetstone" form, and "navicelli" form, which were often seen singly and again in masses. Stroebe was uncertain as to the significance of the cells containing the dark red particles, though he evidently inclines to the opinion that they are parasitic forms. From his plate, which is unfortunately uncolored, it is seen that the safraninophile bodies of various shapes, sometimes occur in the nuclei, and again in the cell protoplasm. In Fig. 7 he shows a cell with a nucleus in mitosis, in the protoplasm of which two darker segments occur with "sickles" or "navicelli" forms. In Figs. 8, 9 and 10, he shows "vacuoles" pushing the nucleus aside and containing in their interior the irregular chromatic particles.

Podwyssozki inclines to the view that the forms described by Stroebe are probably identical with his coccidia, and he believes that since Stroebe did not find the cells filled with "sickle-formed embryos" which he regards as so positively characteristic, he was not able to take a positive stand.

Without going further into a discussion of this subject, which has so many tempting aspects, I will proceed to a brief description of my own studies.

Even before I had made preparations after Podwyssozki's method, I was struck with the resemblance of some of his figures to the safranin-iodine crystals

¹³ Podwyssozki und Sawtschenko. Ueber Paratismus bei Carcinomen nebst Beschreibung eulinger in den Carcinomgeschwulsten schwarotenden Sporozoen, Cent. f. Bak. u. Parasiten., XI, Band, No. 16, 17, 18, 1892.

¹⁴ Stroebe. Zur Kenntniss verschiedener cellularer Vorgange und Erscheinungen in Geschwulsten. Beitr. z. path. Anat. u. z. Allgem. Path., 11 Band, Heft. 1, 1891.

found in normal tissue. A reference to his beautiful plates, VII and VIII, showed in the first place, that many of the nuclei of the epithelial cells contained deep red, irregular masses, quite distinct from the lighter red of the chromatin proper. Among the inclusions, it was noted that the stained masses in their interior, exhibited the peculiar dark red color, and the diversity of shape, so characteristic of the safranin-iodine crystals. Particularly convincing in this respect were the safraninophile particles in the inclusions in Figs. 16, 17, 18, 19, 20, 25, 26 and 27, and also in the masses in the lymph spaces, in Figs. 21, 22 and 24.

I now proceeded to study some sections prepared after the process advised by Podwysoski, employing for this purpose some carcinoma tissue which had been carefully fixed in Flemming's solution. For want of well fixed tissue, it has not been possible for me to extend my experiments as far as seems desirable; but in the half a dozen carcinomata from various sources, and in a well preserved piece of rapidly growing osteo-sarcoma, kindly furnished me by Prof. Van Hook, the results were quite uniform, so that, it seems to me, they should be judged qualitatively, and not quantitatively.

The sections of the paraffin imbedded tissue were fixed on the slide, stained with Babe's anilin-water safranin, washed in picric acid alcohol, cleared in clove oil and mounted in balsam. Other sections from the same pieces were stained in the safranin, and washed with tincture of iodine, followed by alcohol. Others still were stained in safranin, and washed with alcohol acidulated with hydrochloric acid; or they were stained with gentian violet, after Bizzozero's method.

One of the first objects studied was from a carcinoma of the larynx, and in sections of this tumor, prepared as advised by Podwysoski, I was surprised to find in many of the nuclei, particularly at the border of the section, *safraninophile bodies which appeared identical with those produced by the safranin-iodine reaction*. These bodies occurred not only in the nuclei of epithelial cells, but also in the nuclei of the other tissue elements. Their color was the familiar brownish-red of the safranin-iodine crystals, and the size and shape of the bodies varied in the same manner as has been described. In the epithelial cells, the "lancet-like" forms predominated, and these were present either singly or in heaps.

In Fig. 5 is seen a group of epithelial cells from one of the sections of the carcinoma of the larynx, and it gives a good idea of the appearance of a group of cells in which these bodies are plentiful; though it was rare to find so many neighboring cells in a single preparation, showing the safranin bodies.

Fig. 6 shows four cells from the small-celled infiltration, in the same section as the above, two of which present well defined lanceolate safraninophile bodies.

The three large cells in Fig. 7 were found in another section of the same tumor, in a portion which appeared to be degenerating. From their surroundings, I am inclined to regard these cells as pus corpuscles, or other degenerating cells, containing the usual deep red masses. Particular attention should be directed to the shape of the safraninophile bodies in this figure as corresponding with Podwysoski's "button-like," "crescentic," "sickle-like" and "navicelli" particles.

Upon discovering these safranin bodies to be produced by the safranin-picric acid method, I extended the staining method to sections of other carcinomata, and to sections of normal tissue. It was found that while the peculiar red particles were not invariably present in a section stained after Podwysoski's process, they did occur occasionally in the sections, both of carcinomata and of normal tissue. So that it is clearly a fact, that brownish-red safranin bodies of a variety of forms are not found in carcinomata alone, when the safranin-picric acid stain is employed.

For the sake of comparison, sections from the same pieces of tissue as those employed in the last experiments were stained by the safranin-iodine method. Here the usual safraninophile particles were produced, and so far as microscopical appearances were concerned, the resemblance between these crystals and the bodies occurring in the safranin-picric acid stained sections was so great, as to lead me to the opinion that they were similar in nature. That is, that in both cases, the peculiar deep red formations were of a *crystalline nature, and the result of the safranin-iodine or safranin-picric acid reaction*. This opinion gained additional weight from the fact that it was possible to demonstrate a crystalline precipitate when safranin solution and picric alcohol were mixed, directly. It was observed also, in referring to this experiment in another part of this paper, that the production of the precipitate by safranin and picric acid was much more tardy than in the safranin-iodine reaction. This, it seems to me, would explain the preponderance of the safranin-iodine crystals, and the relative scarcity of the crystals of the safranin-picric acid reaction.

I regret not having had the opportunity, up to the present time, of extending my studies over a considerable amount of well fixed carcinoma tissue, because, for this reason, I cannot speak with confidence of a number of Podwysoski's findings in this communication, which therefore, must be regarded in the light of preliminary report. Particularly is this true of the intra-cellular cysts containing safraninophile particles, for I must confess, that my search for such bodies as shown in many of Podwysoski's figures has been poorly rewarded. Nevertheless, the deep red particles of "sickle-form," "spindle-form," "half-moon" form, and other forms described by these authors, and upon which so much stress is laid, bear so great a resemblance to the artificial safranin crystals of my experiments, that I cannot do otherwise than regard them as identical. Another point upon which I lay much stress is the presence of illy-defined deep red particles in the *nuclei of some of the epithelial cells* of Podwysoski's figures, which he has so faithfully portrayed. If one of the deep red intra-nuclear particles were transferred to an intra-cellular cyst, it would make a safraninophile body precisely identical with many of those he has figured. As an example, the deeply stained particle in the nucleus of the epithelial cell in Fig. 17, plate 8, resembles in every way the "sickle-formed" chromatic bodies in the larger inclusion in the same cell.

I have encountered the heap-like collections of cells with safranin particles, which are designated "spore cysts" by Podwysoski, in several of my preparations; particularly in those portions which were degenerating. Such masses I have regarded as leucocytes, and the deep red particles as safranin-picric

acid crystals. Many such leucocytes undoubtedly contain ingested chromatin, from cells which have degenerated. In Fig. 24, plate 8, Podwysoski represents a mass of "sporozoa" occupying the place of an epithelial cell, which, according to his idea, has degenerated. If we recall Noeggerath's¹⁵ observations on the relations of leucocytes to carcinoma, wherein he describes very accurately the manner in which wandering cells take up the dispersed chromatin of degenerating carcinoma cells, and in which he mentions the possibility of leucocytes filling the devoured cell, then this figure of Podwysoski's could be explained. His "sporozoa" would become leucocytes filled with particles of dispersed chromatin, aside from that which belonged to them naturally. This would account for the irregular arrangement and variety of color of the contained safraninophile particles. Aside from this, the fact that these leucocytes contained an excess of chromatin would naturally make them attract more safranin, and thus give them opportunity for developing more safranin-picric acid crystals than ordinary leucocytes.

It has been previously stated, that Podwysoski and Sawtschenko observed that the red blood corpuscles were the only tissue elements which assumed the brownish tint characteristic of the sporozoa, in preparations fixed in Flemming's solution, and stained in anilin-water safranin, and picric-alcohol; but that the absence of safraninophile particles in the red blood cells always distinguished them from the coccidia. In the description of the safranin iodine method, I have referred to the fact that in normal tissue fixed in Flemming's solution, the red blood cells not only were stained a well marked brownish color, but that they also often contained the safraninophile crystals. Extending my studies to carcinoma tissue fixed in Flemming's solution, and stained by the safranin-iodine method, it was often possible to discover red blood cells, both within and without the vessels, of a brownish color, and containing the same safranin bodies observed in the blood corpuscles of normal tissue. Fig. 3, represents a mass of such red corpuscles, and especial attention is directed to the shape and arrangement of the safraninophile bodies, since they resemble so closely some of Podwysoski's coccidia. Two of the corpuscles contain the chromatic particles at the poles, which calls to mind Podwysoski's "direct division" of the sporozoa. If the nature of the cells in this figure were not otherwise clear, the corpuscle (a) at the right of the heap, which presents a profile view with the usual bi-concavity of red blood corpuscles, would remove all doubt.

But the occurrence of safranin bodies, in the red blood cells, was not confined to Flemming fixed carcinoma tissue stained by the safranin-iodine method; for I was also able to demonstrate similar deep red particles in the blood corpuscles of *preparations stained by the safranin-picric acid method*. Fig. 4, from a preparation of an *osteo-sarcoma* of the humerus, fixed in Flemming's solution, and stained in safranin-picric acid alcohol, shows three red blood corpuscles in a connective tissue space, every one of which contains safranin bodies. In this preparation the nuclei of the sarcoma cells showed a brilliant red chromatin network, which made a strong contrast with the deep brown tint of the blood corpuscles, and the

deeper red of their safranin bodies. From these observations it is therefore evident, that the red blood corpuscles, both of the safranin-iodine and safranin-picric acid preparations, *do contain safraninophile substance*; and from my other studies of this subject, I am prepared to assert that the safraninophile substance is an artificial product, and the result of a safranin-iodine, or a safranin-picric acid combination, as the case may be.

This being true, one of the most weighty supports is removed from the arguments of Podwysoski and Sawtschenko. Now, if red blood corpuscles, leucocytes, and pus corpuscles, can contain the foreign safranin bodies, it is not unreasonable to presume that the blood platelets may also become the seat of similar chromatic formations; and if this be true, it is possible to furnish an hypothesis to account for many of the findings of these investigators. Without entirely committing myself, therefore, I desire to suggest, that many of the included cells with safraninophile particles figured by Podwysoski, are red blood corpuscles, or blood platelets, or degenerating forms of these cells which have escaped into the tissue, and which have become the seat of artificial deep red precipitates.

Applying the preceding observations to the work of Stroebe, it does not become difficult to conclude that the intra-nuclear and intra-cellular bodies, which excited his curiosity, are closely related to, or identical with, the safraninophile particles produced in my experiments. This conclusion seems justifiable for several reasons. In the first place, Stroebe employed the safranin-picric alcohol stain in his preparations. Then the bodies described by him are decidedly safraninophilous. Their shape and size recalls the safranin-picric acid particles. Finally, the facts are equally suggestive that these safranin bodies occurred free, both in the nuclear and cell substance, and that they were sometimes single and again aggregated into heaps.

In connection with the recent studies on the structure and etiology of carcinoma, it is interesting to note that the question of the reagent effect has been mentioned by Hansemann.¹⁶ In his latest defense of his theory of asymmetrical mitosis, against the attack of Stroebe, Hansemann objects to certain of Stroebe's observations on the ground that the preparations contained *artificial products* which obscured the karyokinetic pictures. In this condemnation Hansemann includes, also, the preparations of Podwysoski, Coën, and Fischer, which were employed by Stroebe, and he says that while these preparations were doubtless useful for the purpose intended (studies in tissue regeneration) they were not available for studies in irregular mitosis. Now, if the preparations of Stroebe were useless for the study of nuclear phenomena, on account of the presence of foreign products, they certainly should be excluded in the more exacting researches required in determining the nature of the minute cell inclusions, which forms the subject of the work on the carcinoma organism.

The arguments herein presented may be epitomized as follows:

1. A precipitation of deep red material may be produced when the solutions of safranin and iodine, or safranin and picric acid are mixed, directly.

¹⁵ Bietrage zur Structur und Entwicklung des Carcinoms. E. Noeggerath, Wiesbaden, 1892.

¹⁶ Ueber die Anaplasie der Geschwulstzellen und die asymmetrische Mitose. D. Hansemann. Virchow Archiv., Bd. 129, Heft 3, 1892.

2. A precipitation of similar deep red material is produced *in tissue*, when safranin and iodine, or safranin and picric acid are employed in the staining process.

3. The precipitate occurs in any of the elements, either of normal or pathological tissue; and may occur either in the nucleus or in the cytoplasm.

4. This artificial product occurs in the cells of sections of carcinoma tissue, prepared as directed by Podwysoski and Sawtschenko.

5. Therefore, the multiform red particles occurring in the cells of carcinoma tissue prepared by the safranin-picric acid method, are artificial products; and since Podwysoski and Sawtschenko base their arguments on the presence of these red formations in their so-called "coccidia," their conclusions must be valueless.

It is not my purpose to deny that some of the bodies seen by Podwysoski and Sawtschenko, and also by Stroebe, may have been sporozoa. Much less do I intend, in this work, to dispute the presence of coccidia in carcinomatous tissue, or to enter upon the question of the possible etiological relation of protozoan microorganisms to these neoplasms. However, since these investigators employed a micro-technical method, which my experiments lead me to believe is a faulty one, because it gives rise to dangerous foreign products; and since they make the bodies which I believe to be foreign, the decisive issue of this work, it can only remain for me to take an opposing attitude.

It certainly seems an opportune moment to point out the errors of Podwysoski's method; for not only is the work of Podwysoski and Sawtschenko extensively quoted, and credited; but many investigators are straining their eyes to discover the all important lanceolate, crescentic, semi-lunar, falciform, and "navicelli" bodies; and finding these characteristic formations in carcinoma tissue they must confirm the work of these authorities.

To correct a point which may not have been made altogether clear, I desire to state that it is not my intention to throw discredit on *safranin* as a valuable staining substance, for, when followed by a washing with alcohol aciduated with hydrochloric acid, I believe it gives results which all of our knowledge of staining reactions leads us to believe are reliable. But to follow safranin staining with a subsequent washing with a solution containing iodine or picric acid, I regard as not only unscientific, but positively dangerous.

EXPLANATION OF PLATE.

All of the figures were drawn by the aid of the Abbé camera lucida, with a tube length of 160 mm.; drawing paper on the table. The Abbé sub-stage condenser was employed in obtaining illumination.

Figs. 1 and 2 were made with Bausch and Lomb's one-twelfth inch oil immersion objective (Continental Series), and Leitz ocular 4. The remaining figures were all drawn with the Leitz one-twelfth inch oil immersion objective, ocular 4.

Fig. 1. A group of nuclei from the heart muscle of the frog, representing the appearance of the safranin-iodine crystals as first discovered. The relative size and the shape of the bodies is well shown, together with the contrast of their reddish-brown color, with the pinkish hue of the nucleus proper. The nucleus (*a*) is seen in transverse optical section.

Preparation.—Corrosive sublimate, Pfizner's safranin, and tincture of iodine.

Fig. 2. Three red blood corpuscles from same section as Fig. 1. The safraninophile particles are seen in the nuclei

of the corpuscles. The nucleus (*a*) is nearly filled with a finely granular, deep red mass, giving it an almost homogeneous deep red color. In (*b*) only a few safranin bodies are seen. They present a variety of shapes, which doubtless depend upon the attitude from which the bodies are viewed. In the nucleus (*c*) the bodies are larger and more numerous. These three corpuscles represent approximately well, the difference which was often observed in the nuclei of the tissue elements of a single section.

Fig. 3. A group of red blood corpuscles from a carcinoma of the larynx. Illustrating the brownish tint of the corpuscles, and the deep red of the safranin bodies. At (*a*) is shown a corpuscle which presented a profile view, and which exhibited well defined bi-concave surfaces. The safraninophile particles in (*b*), (*c*), and (*d*), cause them to bear a great resemblance to the "direct division" of Podwysoski's coccidia.

Preparation.—Flemming's solution, Babe's anilin-water safranin, and tincture iodine. The tissue had remained some days in the Flemmings solution, and the section exhibited a marked brownish tint from the osmic acid staining.

Fig. 4. From a rapidly growing, medullary, osteo-sarcoma of the humerus, showing three red blood corpuscles in the sarcoma tissue, which are distinguished by their brownish color and their deep red safraninophile particles. A number of blood corpuscles were found exhibiting a similar appearance in various portions of this section, which, from their relations, and resemblance to other corpuscles, left no doubt as to their identity.

Preparation.—Flemming's solution, Babe's anilin-water safranin, and picric acid alcohol.

Fig. 5. A group of epithelial cells from a carcinoma of the larynx (same tumor as Fig. 3). These cells were found near the border of the section, and their nuclei presented a large number and variety of safranin-picric acid bodies. The excess of the "lancet forms" will be noted.

Preparation.—Flemming's solution, Babe's anilin-water safranin, and picric acid alcohol.

Fig. 6. Four cells from the small-celled infiltration of the same section as Fig 5. A variety of deep red safranin particles are seen in the nuclei.

Fig. 7. From a degenerating portion of the carcinoma of the larynx. Three large cells, showing crescentic, semilunar, falciform, "navicelli," and "button-like" safranin formations. These cells were lying free in a granular detritus which contained fragmenting leucocytes, degenerating carcinoma cells, etc. These cells are doubtless degenerated leucocytes (pus corpuscles); and their contained bodies are safranin-picric acid crystals, since they resemble the safranin-picric acid bodies found in other portions of this section.

Preparation.—Flemming's solution, Babe's safranin, and picric alcohol.

In all cases, the tissue employed in this study was cut in small pieces immediately on its removal from the body, and at once dropped in the fixing solution.

IDEAL SURGERY.

Read before the St. Louis Medical Society.

BY G. WILEY BROOME, M.D.,

PROFESSOR PRINCIPLES OF SURGERY AND CLINICAL SURGERY, WOMA MEDICAL COLLEGE; SURGEON TO THE WOMAN'S HOSPITAL; CONSULTING SURGEON TO THE ST. LOUIS CITY AND FEMALE HOSPITALS, ETC.

Mr. President:—It is plainly apparent to the student of medicine that the practice of modern surgery is wholly unlike the practice which prevailed, and was approved, only a few years ago.

In the light of advanced surgical pathology, bacteriology, and the now more familiar processes of regeneration and repair, modern surgical methods bear but slight resemblance to those that were practiced a very short time ago.

It is furthermore clearly apparent that the whole tendency of modern surgery is to secure ideal results, and at the same time to keep the practice well abreast with the revolution necessitated by the indications discovered through intelligent clinical observation, scientific investigation and experimental research.

The scope of this modern ideal surgery—it may