On the Significance of the Plasmal Reaction

BY

A. J. CAIN

(From the Department of Zoology and Comparative Anatomy, Oxford)

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INTRODUCTION

THE 'plasmal reaction' has been widely used in cytology, and is generally interpreted as revealing the presence of aldehydes, but there is considerable doubt as to their source. According to Feulgen and his collaborators, who first described the reaction, the aldehydes (plasmal) are formed by decomposition of acetalphosphatides (plasmalogen) under the catalytic influence of mercuric chloride. According to Verne, they are formed by oxidation at unsaturated linkages in various forms of lipoid, the explanation of the action of mercuric chloride being either that it 'unmasks' the lipoids (Gérard) or that it destroys the inhibiting effect produced by formaldehyde when that substance is used in fixation.

The techniques used for demonstrating the plasmal reaction vary considerably, having in common only the use of Schiff's reagent and the production of results in the cytoplasm and its inclusions, not in the nucleus.

This paper presents evidence to show that the variations in technique are of importance and affect the results. There is no evidence that aldehydes are concerned in the reaction as practised by cytologists, but there is good evidence that oxides and hydroperoxides are the groups responsible in many cases, and that although acetal linkages will give rise to aldehydes under the conditions of the improved technique suggested, there is no *histochemical* evidence that such linkages are in fact present in the tissues investigated. Such substances have been prepared from tissues by Feulgen and his school, but the plasmal technique as usually practised does not show them clearly.

COMPARISON OF TECHNIQUES

The work of Feulgen and his school has been reviewed from the biochemical standpoint by Feulgen and Bersin (1939) and completed by the synthesis of an acetalphosphatide (Bersin *et al.*, 1941). Imhäuser (1927) carried out a

[Quarterly Journal Microscopical Science, Vol. 90, part 1, March 1949]

survey of the occurrence of plasmal in tissues, and concluded that it was very widespread indeed. The technique was described by him and by Feulgen and Voit (1924).

Verne has been the principal investigator of plasmal and has modified the technique. He considers that positive results are due to the production of aldehydes by the atmospheric oxidation of double bonds (1928*a*, *b*, *c*, 1929*a*, *b*, 1936*a*, *b*, 1937*a*, *c*). Lison (1932) showed that Schiff's reagent would react with oleic acid and certain other unsaturated substances besides aldehydes. He noticed that not all of these would react with other compounds used to characterize aldehydes, nor did a reaction with one of these compounds mean that the substance would react with others. Oxidant enzymes might also recolour Schiff's reagent. Gérard (1935) believed that, myelin excepted, a positive result was always due to 'oxidases'. Lison (1936*a*) showed that weak positive results with the Nadi reagent were due to peroxides formed in lipoids by atmospheric oxidation. Verne (1937*b*, 1940) disagreed with these conclusions and reaffirmed his theory that aldehydes were responsible.

The techniques used were as follows. All employ sections cut on the freezing microtome.

1. *Technique of the Feulgen School*

Frozen sections of fresh tissue are used, some being treated with mercuric chloride solution, the rest acting as controls. Untreated sections negative or only weakly positive with Schiff's reagent, treated sections intensely positive.

Since this is the technique prescribed by Feulgen, it alone can be called the plasmal technique.

2. *Technique of Verne*

Tissues are fixed in a mercuric chloride or platinum chloride fixative. No control section.

Lison (1933) referred to this as the Feulgen–Verne technique (F.V.), a name usually employed by Verne himself.

3. *Technique of Gérard*

Tissues are fixed in a formaldehyde-fixative which is carefully washed out. A control section is used, which is left in distilled water while its fellow is in mercuric chloride solution.

4. *Technique of Guyon (1932)*

This appears to be the plasmal technique as applied to myelin. Both Guyon and Verne refer to positive results with fresh tissue. This does not, apparently, mean that fresh tissue will recolorize Schiff's reagent. A short treatment with mercuric chloride is necessary. However, Verne (1937*a*, p. 4) does seem to refer to a positive result without mercuric chloride.

Most investigators have used preliminary treatments with phenylhydrazine or semicarbazide or their derivatives to prevent the reaction. Sodium

bisulphite and dimedone have also been employed. But these will react with compounds other than aldehydes, and most are strong reducing agents which might be expected to prevent atmospheric oxidations. Their value in histochemistry is discussed below (p. 82).

Experimental Results

The following questions arise from the preceding discussion:

What is the relation between the results obtained by the various techniques?

How far can the phenomena observed be reproduced with unsaturated lipoids *in vitro*?

Is the technique entitled to rank as a histochemical test?

They will be dealt with in the order given.

A. *The Relations between the Various Techniques proposed*

Feulgen's original technique—the plasmal technique as it will be called in this paper—was tried on mouse testis, which should show a positive result in the interstitial cells and a negative or very weak one elsewhere. Frozen sections of fresh tissue were stuck on to slides coated with albumen by gentle and rapid heating; some were treated with mercuric chloride solution, others passed directly into Schiff's reagent. After 10 minutes the slides were washed carefully in sulphur dioxide water, as prescribed by Verne (1929*a*, repeated by Lison, 1936*b*, p. 216), to remove all traces of Schiff's reagent and so prevent the subsequent reformation of basic fuchsine. Sections were also used which had not been subjected to heat.

This technique is unsuitable for all those tissues that will not retain their coherence on thawing after sectioning. The control sections, both heated and unheated, showed a very faint purplish-red coloration throughout, which was also seen in places where there was an appreciable amount of albumen. Schiff's reagent is very difficult to wash out thoroughly, even from sections, and this general coloration is considered to have no significance. The sections treated with mercuric chloride solution showed a much deeper, patchier, and rather bluer coloration, but were too badly smeared to allow its precise location to be determined.

The Feulgen–Verne technique was tried on the same material, but fixation was in saturated mercuric chloride solution, that concentration being considered more suitable, and was for 6 hours only. Treatment with Schiff's reagent gave a positive result which was more feeble than that obtained by the plasmal technique. Gérard's technique, formal-calcium (Baker, 1944) being used as fixative for 6 hours, gave an extremely feeble, almost negative, result with the control sections, and a positive intermediate between the plasmal and Feulgen–Verne results with the sections treated with mercuric chloride.

Adrenals of mature male and immature male and female white rats were used for a more extended trial of Gérard's method. It was found that after

6 hours' fixation, the medulla was almost negative; the cortex showed a positive result only in the zona glomerulosa and the innermost region of the zona reticularis; these results were exceedingly feeble and the intensification with mercuric chloride was very slight indeed. But sections left overnight in water showed a much more positive result in the cortex, which appeared to follow the distribution of lipoids very closely, being most marked in the outer region of the zona fasciculata where the cells are very heavily laden. The picture seen was exactly comparable with those obtained on the same material with Sudan IV or Sudan black (Harrison and Cain, 1947, pl. I, fig. 2). There was no visible difference between the controls and treated sections. The Feulgen–Verne technique (with fixation for 6 hours) gave the same results except that the sections were brighter throughout, and the medulla was as bright as the cortex. Sections left overnight in water showed a considerable intensification of the colouring in the cortex, and a slight reduction in the medulla.

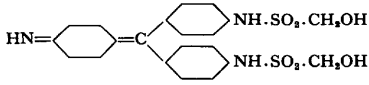
As frozen sections of fresh tissue were of very little use, observations were made macroscopically on small pieces of tissue. Portions of mouse skin, body-wall, liver, kidney, testis, and adrenal were dropped into Schiff's reagent. Colouring was seen only after 20 minutes, and then principally at the cut surfaces. It spread and intensified slowly over a period of hours. But pieces of the same tissues dropped into Schiff's reagent containing mercuric chloride behaved very differently. An intense reaction was seen throughout the tissue (except in the case of liver and of adipose tissue) within 5 minutes, and it had apparently reached its maximum within 15. The colour produced was a very deep violet. This reaction differs remarkably in speed, colour, and intensity from those previously seen.

The relation between this intense reaction and that obtained on fixed tissues was investigated by repeating the experiment with mouse skin, body-wall, kidney, and liver (to act as control), after various periods of fixation in mercuric chloride and in formal-calcium, and comparing the intensity and shade of the resulting coloration. It was found that after 2 hours' fixation in mercuric chloride solution, the reaction was definitely weaker. After formal-calcium, followed by an hour's washing in running water to remove the formal-dehyde, only a feeble reddish-purple coloration was produced. After 2 hours in the reagent (Schiff plus mercuric chloride) both lots of tissue were considerably more intensely coloured, though by no means as dark as the unfixed tissue, and the formaldehyde-fixed material was still somewhat redder, though much less so than at first. After 6 hours' fixation, the mercuric chloride-fixed pieces showed a coloration slightly paler than that obtained after 2 hours; liver was slightly more coloured than before. The formal-dehyde-fixed pieces were much weaker. After 24 hours, the reaction was not prevented but was comparatively very weak, and there was a strong tendency for the liver to colour.

It would seem, then, that there are two distinct phenomena involved. On the one hand is the intense and rapid reaction given by fresh tissue treated

with a mixture of Schiff's reagent and mercuric chloride, which is progressively reduced by fixation, and apparently affected specially by formaldehyde. On the other is the reaction seen in adrenal cortex, slower, increasing with time, obviously closely connected with the amount of lipoid present, and affected comparatively slightly by treatment with mercuric chloride. It appears to be this reaction that is shown by the techniques of Feulgen–Verne, and of Gérard, if fixation is prolonged (more than 3 hours), with a trace of the other if treatment with Schiff is carried out on sections not more than 4 or 5 hours after the tissue is placed in the fixative.

As it is so difficult to wash out Schiff's reagent, and previous fixation is not to be recommended for studying the rapid reaction, an observation on the solubilities of Schiff-compounds (further discussed below) was used in an attempt to improve on the plasmal technique. The compound between formaldehyde and Schiff's reagent is water-soluble and, as will be seen from its formula,



it is a very feeble dye. The following technique was therefore employed:

1. Drop pieces of fresh tissue into a mixture of equal parts of
   (*a*) Schiff's reagent diluted with its own volume of sulphur-dioxide water, and
   (*b*) a saturated aqueous solution of mercuric chloride.
   For controls, drop other pieces into the diluted Schiff's reagent, without addition of mercuric chloride.
   Allow the pieces to remain in these solutions for 15 minutes.
2. Remove tissues, wash in sulphur-dioxide water to remove the excess Schiff's reagent without allowing recoloration, and drop immediately into formalin (40 per cent. formaldehyde). Leave for 2 hours.
   The formaldehyde-Schiff compound forms rapidly, and the control pieces become almost as dark violet as the others.
3. Wash in running water for 2 hours or more until the control pieces have become only a very pale lilac. This washing also removes the formaldehyde before embedding.
4. Either cut sections without embedding, or embed in gelatine overnight, harden in formalin, cut sections, and mount in Farrants's medium. As the colour begins to pale, these operations should be carried out as quickly as possible.

This method is applicable only to very small pieces of tissue, as penetration by Schiff's reagent is poor. The results obtained were good, the stain being very intense and apparently very selective, especially in skin. It was noticeable that fat droplets were completely negative but the cytoplasm of the fat-cells was heavily positive. Sebaceous glands were negative, the bases of the hair

follicles feebly positive, muscle and myelin sheaths very positive. Remarkably enough, small elements scattered in the connective tissue are feebly positive; these appear to be nuclei of connective tissue cells. The nuclei of the fat-cells also appear to be coloured faintly. A control section should therefore be coloured with Sudan black, to demonstrate the lipoids present, and only positive results obtained on lipoids should be considered. In mouse adrenal, the medulla was negative, the cytoplasm of most of the cortical cells positive, the lipoid droplets completely negative. The control sections were colourless in all cases.

Sections prepared by the Feulgen–Verne technique showed a much more feeble coloration or none at all.

B. *Experiments with Unsaturated Lipoids* in vitro

Oleic and linoleic acids were available for these experiments. In general, it was found that, as might be expected, linoleic acid reacted more rapidly and gave more intense results than did oleic acid. Presumably arachidonic acid would be more reactive still. In interpreting the results it must be remembered that pure lipoids are well known to be much more slowly oxidized than impure ones, and that lipoids in tissues may well react far faster than the same substances when extracted.

Both oleic and linoleic acids will react with Schiff's reagent after about an hour, producing a fine purple, which is certainly not the colour of basic fuchsine. It is worth noting that if a thin layer of oleic acid is allowed to lie on the Schiff's reagent in a test-tube without disturbance, the colour is formed at the interface and shows a strong tendency to diffuse in the aqueous fluid, the rest of the oleic acid being uncoloured. If, however, the tube is continually shaken, the oleic acid gradually becomes coloured, and the Schiff's reagent is only slightly tinged. The same effect can be seen with linoleic acid.

Both oleic and linoleic acids if exposed to the air will become coloured by Schiff's reagent within 15 minutes. After prolonged exposure (several days) coloration takes place within 2 or 3 minutes. Linoleic acid solidifies as it oxidizes, oleic acid does not. Strips of filter-paper soaked in the acids and exposed on clock-glasses protected from dust are convenient for these experiments. If they are exposed to mercuric chloride solution, oxidation is very much more rapid. Linoleic acid exposed to the air for 18 hours colours with Schiff's reagent in 10 minutes. If left in contact with mercuric chloride for that length of time, it colours almost instantaneously. After treatment with saturated mercuric chloride solution for 15 minutes the unoxidized acids showed no shortening of the time taken to colour with Schiff's reagent. Partly oxidized acids showed a very slight shortening and intensification of the colour. The extremely high rate of oxidation of the lecithins is well known, and arachidonic acid is particularly associated with them (Hilditch, 1947). It is possible that a pseudoplasmal reaction might be caused through acceleration of the oxidation of highly unsaturated acids by mercuric chloride.

Unoxidized oleic and linoleic acid can be prevented from oxidizing by treatment with phenylhydrazine, 2–4 dinitrophenylhydrazine, and semicarbazide. The oxidized acids recolour Schiff's reagent in the same time as do samples of oxidized acid untreated with these reagents. As neither the pure nor the oxidized acids will reduce ammoniacal silver nitrate even on boiling, it appears that aldehyde groups are not formed during atmospheric oxidation for at least a week, and that phenylhydrazine and similar reagents, being reducers, can block further oxidation by their presence, as do carotenoids dissolved in fats. It was noticed that the acids became darker and redder in colour, indicating that the phenylhydrazine was being oxidized.

In contrast to the above, palmital and stearal, after treatment with phenylhydrazine, were completely negative to Schiff's reagent, and remained so after immersion in it for 24 hours. The phenylhydrazone was not decomposed by the acidity of the reagent within this period.

Verne (1929*b*) remarks that by treatment with phenylhydrazine for 24 hours, the staining is abolished in both the cortex and medulla of the adrenal. This is confirmed for the adrenal of the rat. Staining in the medulla is discussed below.

C. *The Status of the Plasmal Reaction as a Histochemical Test*

Danielli (1947) has shown that the product of the reaction between Schiff's reagent and desoxyribosenucleic acid is water-soluble, diffusible, and readily taken up by chromosomes when used as a stain, the rest of the preparation being coloured faintly. The colour produced with oleic and linoleic acids is diffusible, as mentioned above, in the Schiff's reagent. Slices of an oleic acid-gelatine emulsion fixed in formaldehyde and carefully washed were coloured and placed in various solutions. The colour appears to be slightly soluble in very dilute acids, more so in stronger ones, but insoluble in alkaline solutions and in alcohol.

The coloured substance produced from Schiff's reagent and acetaldehyde is very slightly soluble in distilled water, insoluble in alkaline solutions, and very slightly in acid ones, but readily soluble in alcohol. It is a very deep violet. The substance produced from Schiff's reagent and formaldehyde is easily soluble in distilled water and dilute acids and alkalis, but only slightly in alcohol. That produced from palmital and stearal appears to be insoluble in water, dilute acids, and dilute alkalis, but easily soluble in alcohol. In aqueous suspension it shows no dyeing power.

The properties of the formaldehyde-compound were further investigated. Sections of mouse skin placed in an aqueous solution became violet but lost all their colour on washing, except that the hair-shafts remained very faintly purple for some time. Sections of rat adrenal took up the colour on prolonged immersion, the heavily laden fat-cells in the cortex being coloured most deeply. The medulla was scarcely tinged. Immersions up to an hour produced almost no effect. Also, as mentioned above, the colour even when produced *in situ* can be washed out completely.

It appears, then, that while the lower members of this series may be water-soluble in certain circumstances, the higher ones are not, and none have any appreciable tinctorial power. The slightest contact with regenerated basic fuchsine is far more dangerous than an hour's soaking in a solution of one of these substances; fortunately, the colour produced is a very light red, and stains almost all parts of a section indiscriminately. However, as Lison has shown, colour is not always a trustworthy guide. But if one section in a series is coloured much more red (less blue) than others, it should be regarded with suspicion.

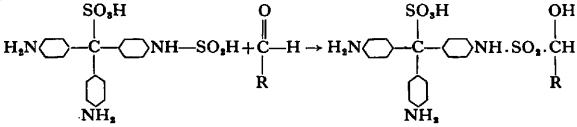
Discussion

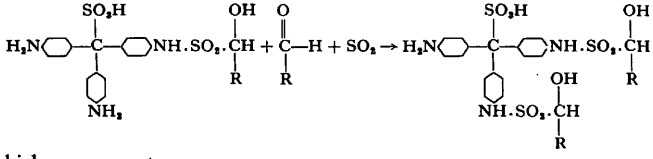
It appears that in the past two distinct reactions have been confused under the name of 'plasmal', the intense rapid initial coloration, which is apparently due to 'plasmal' in Feulgen's sense, and the slow reaction, increasing with passage of time and only slightly affected, if at all, by mercuric chloride, which is due to autoxidation products of unsaturated lipoids.

The first, the plasmal reaction, is seen at its most intense when fresh tissue is acted upon by a mixture of Schiff's reagent and mercuric chloride. Schiff's reagent by itself showed no positive result within 15 minutes, or only an exceedingly feeble one, on the tissues examined. This reaction is, as Imhäuser showed, very widespread. Liver is almost negative, but the other tissues examined show intense colorations. Fixation reduces the intensity of the reaction progressively, and it is interesting to note that pieces of tissue give a more intense reaction than do sections cut without embedding from the same pieces before treatment. Palmital and stearal polymerize very readily, and are, in general, very reactive substances. Presumably the increased exposure consequent upon section-cutting assists in their alteration.

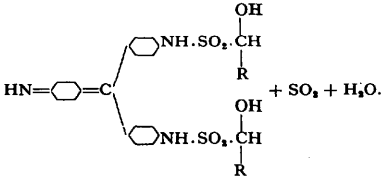
Fat globules are totally unaffected by this reaction. In the other, they appear to be the principal sites of it, and stain intensely in the cortex of the adrenal in sections which have been left in water overnight. Verne considers the resulting coloration as a positive result for plasmal. But the distribution of the colour has altered considerably, and the characteristics of the reaction are quite different. It increases in intensity with the passage of time, and mercuric chloride has little or no effect. The work of Gérard and of Lison shows that oxidizers appear in connexion with this reaction in certain cases at least. This is in agreement with part of the chemical evidence on the autoxidation of unsaturated lipoids, during which process many and varied radicles may appear (Markley, 1947; Hilditch, 1947). If it is known that one is dealing with a single substance, or even with two, then possibly they may be identified by the use of a large number of reagents undergoing different types of reaction. In this particular case the number of radicles is unknown, and the reactions available appear to fall into only three groups—reduction, addition, and condensation, as exemplified by ammoniacal silver nitrate, sodium bisulphite, and phenylhydrazine. The reaction of aldehydes with Schiff's reagent is an

addition followed by a condensation, according to Wieland and Scheuing (1920):





which rearranges to:



but as Lison has shown, oleic acid can react, as the elements of water are supplied entirely from the Schiff's reagent. The reaction with silver nitrate, which, as Verne remarks, is not easy to carry out on sections, is also given by ethylene oxides, and addition reactions can also take place across carbon-carbon double bonds; and although phenylhydrazine and similar compounds appear to be specific for the $> C = O$ group, their reducing-power can interfere with the reactions of groups other than aldehydes and ketones. Sodium bisulphite and dimedone (Heilbron and Bunbury, 1943) are also reducing agents. In these circumstances no certain conclusion can be drawn as to the composition of reacting mixtures. The only conditions under which any definite statement could be made about reactions in tissues are found when the reaction can be reproduced *in vitro*, and analysis of the extract shows that only one substance is responsible; these conditions have never been satisfied.

In the case of the plasmal reaction, there is one other characteristic, which is of the utmost importance, namely, the speed at which the reaction is produced under the influence of mercuric chloride. It has been shown above that the speed of autoxidation of unsaturated fatty acid radicles can be greatly increased by the presence of mercuric chloride, the normal and the catalysed rates both increasing with the degree of unsaturation. Studies on arachidonic acid are unfortunately not available, and the possibility should be borne in mind that a short treatment with mercuric chloride of fresh tissues containing

it might cause an appreciable intensification with Schiff's reagent. But the speed of breakage of the acetal linkage is so great, as Feulgen showed, that it seems reasonable to treat positive results obtained within 10 minutes on sections or small pieces of tissue as being due to the liberation of plasmal. However, there is no absolute certainty. A prolonged treatment with Schiff's reagent must not be used because the oleic and other unsaturated radicles might begin to react directly.

The evidence that this reaction, produced rapidly under the influence of mercuric chloride, is due to the liberation of palmital, stearal, and other aldehydes from acetalphosphatides, is entirely the work of Feulgen's school, and its interpretation in that sense rests entirely upon this basis. Consequently, in carrying out the reaction care must be taken that the necessary conditions are complied with. Tissues should be handled rapidly to prevent autoxidation, and a control section is essential because, as has already been remarked, a positive result upon fresh tissue untreated with mercuric chloride might be due to any or all of several radicles and the means at our disposal do not allow of a discrimination between them.

This raises an interesting point. Unless the substrate can be shown to be lipoid, there is no justification for considering the reaction to be due to plasmalogen and plasmal. A case in point is the medulla of the adrenal. The extremely powerful lipoid-colorant Sudan black shows no lipoid whatever in this region. Yet it colours, after Verne's technique, as strongly as the innermost region of the zona reticularis and the outermost of the cortex. Prolonged exposure of sections does result in the cortex becoming much more positive, but the medulla becomes more pale. Obviously, this is not a case of the Feulgen–Verne reaction of unsaturated lipoids; if it were, the medulla also would become more positive. But equally it cannot be said to be a true plasmal reaction because the medulla is negative with the technique given above. As Verne remarks (1929*b*), phenylhydrazine abolishes the reaction in both cortex and medulla. But phenylhydrazine is a strong base, and soaking tissues in strong bases reduces considerably their power of being stained by basic dyes. If this were the mechanism, then the nature of the colouring matter is in doubt, because it has the appearance of a product of Schiff's reagent, but, as we have seen, such products do not appear to act appreciably as dyes. Verne claims that it is due to aldehydic substances which diffuse from the cortex into the medulla. The complete proof of this would require that a definite reaction could be produced on extracts of the medulla made with lipoid-solvents, and that this could be shown to be the same as that found in the reticularis. A mere suppression of the reaction by treatment with alcohol is not sufficient proof. Alcohol has many effects on tissues besides extracting some of the lipoids. There is, however, another possible explanation. Basic precipitates of mercuric chloride solutions will turn deep violet in the presence of Schiff's reagent. Now the colouring of adrenal sections fixed in mercuric chloride is brightest around the free edge of the cortex and the edges of the large fenestrae caused by vigorous fixation in the medulla. It is at least

possible that this distribution is due to unequal conditions of fixation. One may decide that these explanations are unlikely, and it is more reasonable to attribute the reaction to plasmal; but there is no histochemical proof. But where lipoids can be demonstrated in the tissue, and the reaction ceases when they are removed, and mercuric chloride exerts a profound and rapid effect, it is likely that plasmalogen and plasmal are responsible.

The techniques available for demonstrating plasmal histochemically are not completely satisfactory, the demands of the cytologist and chemist being at variance. The reaction can easily be studied macroscopically on small pieces of fresh organs dropped into the reagents. Short fixation is necessary for good results. As mercuric chloride is a very powerful precipitant, the best procedure is probably to fix very small pieces for a short time in a formaldehyde fixative, wash carefully, and carry out the technique on frozen sections in the way usually recommended. But pieces fixed and stained simultaneously according to the procedure given above should also be examined. The reaction in them is far more intense, and far more tissue elements are coloured. This is almost certainly the reason why the Feulgen school, working on fresh sections, found the plasmal reaction so widespread, while Verne, using fixed material, found it far less so. As liver appears to give only the feeblest reaction even when fresh, a piece of it can be carried through with the other tissues under investigation, to act as a general control on the method. It is not denied that a true plasmal reaction can be obtained on pieces fixed for several hours, but it is greatly reduced and may easily be confused with the beginnings of the Feulgen–Verne reaction.

ACKNOWLEDGEMENTS

I have to acknowledge the kindness of the Arthol Manufacturing Company, Chester, who presented me with specially prepared specimens of palmital and stearal. Acknowledgements are also due to Dr. R. G. Harrison for permission to quote work on rat adrenals originally carried out in collaboration with him in the course of other experiments.

SUMMARY

1. Two different reactions have been confused under the name 'plasmal'. One, the true plasmal reaction, is due, as its discoverer Feulgen showed, to the liberation of higher aliphatic aldehydes from acetalphosphatides, the reaction taking place very rapidly under the influence of mercuric chloride, the products being coloured deeply with Schiff's reagent. The other reaction is due to the oxidation, by atmospheric oxygen, of double bonds in unsaturated fatty acid radicles. It is affected only slightly, if at all, by mercuric chloride, and increases with exposure of sections or pieces of tissue to atmospheric oxygen.

2. There is no completely satisfactory method for the histochemical demonstration of plasmal. Sections of fresh tissues are not usually suitable for cytological work, and fixation causes a reduction in intensity of the

reaction. Also, the permissible fixatives are not all of the highest quality. Short fixation in a formaldehyde fixative (e.g. formal-calcium) followed by careful washing is probably the best, but the results should be compared with those obtained by the direct reaction of small pieces of fresh tissue. A suitable technique is described.

REFERENCES

BAKER, J. R., 1944. Quart. J. micr. Sci., **85**, 1.
BERSIN, T., *et al.*, 1941. Zeit. physiol. Chem., **269**, 241.
DANIELLI, J. F., 1947. In *Nucleic Acid*, Symp. Soc. exp. Biol., no. 1, 101.
FEULGEN, R., and BERSIN, K., 1939. Zeit. physiol. Chem., **260**, 217.
—— and VOIT, K., 1924. Pflügers Arch., **206**, 389.
GÉRARD, P., 1935. Bull. Histol. appliquée, **12**, 274.
GUYON, L., 1932. C. R. Soc. Biol., **109**, 1101.
HARRISON, R. G., and CAIN, A. J., 1947. J. Anat., **81**, 286.
HEILBRON, I. M., and BUNBURY, H.M. (edit.), 1943. *Dictionary of Organic Compounds*, vol. 1. London (Eyre & Spottiswoode).
HILDITCH, T. P., 1947. *The Chemical Constitution of Natural Fats*, 2nd ed. London (Chapman & Hall).
IMHÄUSER, K., 1927. Biochem. Zeit., **186**, 360.
LISON, L., 1932. Bull. Histol. appliquée, **9**, 177.
—— 1933. Ibid., **10**, 238 and 292.
—— 1936*a*. Bull. Soc. chim. Biol., **18**, 185.
—— 1936*b*. *Histochimie animale, Méthodes et Problèmes*. Paris (Gauthier-Villars).
MARKLEY, K. S., 1947. *Fatty acids. Their Chemistry and Physical Properties*. New York (Interscience Publishers).
VERNE, J., 1928*a*. C. R. Ass. Anat., **23**, 465.
—— 1928*b*. Bull. Soc. Neur., **1**, 722.
—— 1928*c*. C. R. Soc. Biol., **99**, 266.
—— 1929*a*. Ann. Physiol., **5**, 245.
—— 1929*b*. Arch. Anat. micr., **25**, 137.
—— 1936*a*. C. R. Soc. Biol., **121**, 609.
—— 1936*b*. Bull. Histol. appliquée, **13**, 433.
—— 1937*a*. C. R. Ass. Anat., **32**, 1.
—— 1937*b*. Bull. Histol. appliquée, **14**, 269.
—— 1937*c*. Bull. Ass. Anat., no vol. number, 1.
—— 1940. C. R. Soc. Biol., **133**, 75.
WIELAND, H., and SCHEUING, G., 1920. C. Ber. deut. Chem. Ges., **54B**, 2527.