2. It is concluded that such a porphyrinuria does not derive from an abnormal breakdown of the prosthetic group of haemoglobin, but in some other way. Dietary deficiences are thought to be responsible for the failure to observe this phenomenon in the present experiments.

3. The increase in faecal excretion of the urobilins was not noticed in all the rats treated with the drug, but in most there was a marked increase in the ratio of mesobilene-*b* to tetrahydromesobilene-*b*. The latter phenomenon is probably due to the influence of the drug on the flora of the intestines.

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Acid-soluble Pigments of Shells

1. THE DISTRIBUTION OF PORPHYRIN FLUORESCENCE IN MOLLUSCAN SHELLS

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Red fluorescence in marine shells was described during the last century by McMunn (1886), and later by Furreg & Querner (1929), who drew attention to the importance of fluoroscopy as an aid to systematics. In 1930 the first of a series of observations by Fischer and his co-workers (Fischer & Jordan, 1930, 1934; Fischer & Hoffmann, 1937) dealt with the isolation from shells described as *Pteria radiata*, one of the group of pearl mussels, of a porphyrin intermediate in character between uroporphyrin and coproporphyrin. This substance was named conchoporphyrin. Uroporphyrins were isolated from other species of *Pteria* (Fischer & Haarer, 1931; Waldenström, 1937; Tixier, 1945), and from a species of *Clanculus* and several Trochidae, whose fluorescence had been observed by Querner (Tixier, 1945). Fischer had noticed the absence of red fluorescence in *Helix pomatia*, but since Tixier's paper no serious attempt seems to have been made to determine the exact systematic range of shell porphyrins among the mollusca. A study by Turek (1933) details a wide range of fluorescent colours, some at least of which do not appear to be true examples of fluorescence, but the only other study of molluscan porphyrins in recent years is that of Dhéré & Baumeler (1928) on the dermal porphyrin detected by McMunn (1886) in *Arion*.

In the present investigation an attempt was made to ascertain by fluoroscopy the extent of the tendency throughout the mollusca to deposit shell por-

phyrin. A serious criticism of the work of Fischer arises from the inadequacy of his identification of the species under study—much of his investigation was based on polished and unlocalized material. In the case of *Pteria radiata* in particular, a member of a genus which has been heavily overdescribed, identification of cleaned shells is almost impossible. The differences between the porphyrins isolated from this species and from related forms render a higher degree of systematic accuracy essential. We do not claim to have overcome this standing difficulty. The present survey covers the whole field of molluscan shells, and the help given us by experts in the various groups does not wholly compensate for a lack of detailed identification. As an arbitrary measure of reliability, throughout this communication specific names marked * are based upon the tablet names in the Natural History Museum: unmarked names are derived from material in private collections, for the identification of which we must accept responsibility.

EXPERIMENTAL

Criteria of porphyrin deposition

The specificity of red fluorescence in molluscan shells, on which we relied as a primary guide to the presence of porphyrin, rests on fairly firm ground. Reddish fluorescence of non-porphyrin origin has been mentioned in connexion with only four molluscan pigments, in *Haliotis californiensis* (Lemberg, 1931), in a phase of 'haliotirubin' (Lederer, 1940) which may be the same material; in the blue phase of chromodorin, which shows 'slight reddish fluorescence' (Crozier, 1916), and in aplysiopurpurin (Derrien & Turcini, 1925). Pink and orange fluorescence found by us in several species is described below.

Porphyrins contained in shell are at least as stable as the visible shell pigments, and old museum material has the advantage of being free from non-specific emission due to muscle and fixatives. Much of the material in the Natural History Museum is upwards of 100 years old, but intense red fluorescence was obtained in many instances, and its brightness was fully equal to that found in fresh material of the same species. Marked porphyrin fluorescence was found in specimens of *Gibbula cineraria* from the Clyde beds (post-Pleistocene), in *Pteria media* from the London Clay, and in several species from the Calcaire Grossier (Paris basin, upper Eocene)—*Fissurella squamosa* Desh., *Angaria calcar* Lk., *A. lima* Lk., and *Tectus crenularis* Lk. In these four species no visible pigment whatever remained.

Methods

Shells of about 3000 species, including pelecypods, land and fresh-water gastropods and a few scaphopods, were examined under an 80 W. Osira mercury arc mounted in a Wood's glass envelope. The amount of visible light passing this filter was insufficient to obscure the fluorescence in any but the most highly polished forms. Following the experiment of Fischer in direct spectroscopy of the shell, we also examined a number of species, both those found to be fluorescent and others showing non-fluorescent pigments of various kinds, by this method. A 100 W. opal lamp was mounted in a box about 3 cm. below a black metal screen in which a small hole had been made. This screen carried a glass sheet to protect the specimen from heat, and over the hole was mounted a Hartridge reversion spectroscope (Beck). In the case of very rough shells, it was occasionally found necessary to mount the specimen on the arm of a 50-cycle buzzer, in order to secure a uniform field. The presence of porphyrin bands was taken as confirmatory evidence.

Where porphyrins were found, and material for sacrifice was available, acid extracts of the shell were made and examined directly by means of the reversion spectroscope, and a Hilger quartz ultraviolet spectrograph, using a ribbon-filament lamp as source. Details of the separation and character of the pigments found will appear in a separate paper.

RESULTS

Two main shades of porphyrin emission, a scarlet and a paler eosin-pink, were detected, the second being usually found in thin species, but both being present in *Aplustrum*. Beside these typical porphyrin shades, we noticed several other colours of fluorescence, usually of low intensity. Nacreous shell usually fluoresces blue, and adductor scars white. Yellow tints are often due to the presence of varnish, but true yellow emission occurs in some examples of *Proserpina nitida*, and in the pedal slime of *Helix aspersa* (Turcini, 1926). Several species of *Cypraea*, especially the orange-brown variant of *C. tigris*, show orange emission, and we found a similar colour in the lip of *Papuina boivini*, in the mark corresponding to the visceral hump of *Patella depressa*, in *Alcadia* and *Tellina* spp. and elsewhere. The eggs of *Subulina* have an intense white fluorescence, and can be seen through the parent's shell.

Distribution of porphyrins in marine genera

Porphyrin deposition in marine molluscs centres around the following genera, in which it is widespread.

Gastropoda	Pelecypoda
Fissurellidae	*Placuna, Enigmonia*
Trochidae (many genera)	*Pinna*
Angaria	*Pteria, Pinctada*
Leptothyra	*Isognomon*
Lithopoma	*Vulsella*
Tricolia	*Malleus*
Erato	
Trivia	
Hydatina	
Bulla	
Aplustrum	
Haminoea	
Umbraculum	

In the following additional groups, the occurrence of shell porphyrin is sporadic. The number of species in which it was detected is added, a star (*) indicating that there are probably others.

Gastropoda		Pelecypoda	
Acmaea	1*	*Anomia*	1
Torinia	1	*Clausinella*	1
Cypraea	6	*Gafrarium*	1
Marginella	3	*Sunetta*	1
Velutina	2*		
Actaeon	1*		

Beside these, a few species of *Theodoxus*, a freshwater genus closely related to the marine Neritidae, produce traces of shell porphyrin. Among gastropods, there is a focus of porphyrin deposition in the primitive groups of marine forms (Archaeogastropoda), with another among tectibranch opisthobranchs, and another in an isolated group including *Erato*, *Trivia*, *Velutina*, and a few *Cypraea*. Whereas of 100 odd species of *Cypraea* and fifty of *Marginella*, only six and three species respectively were found to deposit porphyrin, almost all the species of *Trivia* examined (except *T. oniscus* and *T. nivea*) are regularly fluorescent. The trochid group shades off into forms related to the Turbinidae, and it is already known, from work by Krukenberg (1883), Tixier (1945), and others, that linear tetrapyrroles (bilitrienes) play a part in the pigmentation of these shells. Perhaps at this point in molluscan phylogeny the power to open the porphyrin ring was developed. Porphyrins are frequently found in those gastropods whose shell is partially enveloped in life by the mantle lobes. In *Cypraea* particularly, the porphyrin seems to be associated with those parts of the pigmentary pattern which are laid down after the formation of the lip. In addition to the distribution in gastropods, which is set out in greater detail in Table 1, traces of pink fluorescence were noted in the scaphopods *Dentalium entalis*, *D. formosum*, and the Loricates *Ischnochiton herdmanni* and *Tonicia ceylonica*.

Table 1. *Porphyrin fluorescence: distribution in marine species—gastropoda*

(S = Scarlet; E = Eosin pink.)

	Emission tint	Conformity with pigment pattern	Site	Intensity
* *Clypidina notata*	S	Yes	Tip and interior	Medium
* *Fissurella peruviana*	S	Yes	Rays and interior	Medium
F. maxima	S	Yes	Rays, bevel of lip	Strong
F. pulchra	S	Yes	Rays, bevel of lip	Medium
* *F. latemarginata*	S	Yes	General	Very strong
Lucapina crenulata	S	No	Diffuse	Medium
Acmaea virginea	E	Yes	Rays	Weak
* *Trochus niloticus*	S	Yes	Red areas, apex	Medium
* *T. sandwichiensis*	S	?	Red areas, apex	Medium
* *T. pyramis*	S	No	Area on columellar lip	Strong
Clanculus puniceus	S	Yes	Red areas	Strong
C. clangulus	S	Yes	Purple areas	Medium
* *C. pharaonius*	S	Yes	Red areas	Medium
C. floridus	S	Yes	Red areas	Very weak
* *Ethalia guamensis*	S	Yes	Umbilicus	Strong
* *E. striolata*	S	Yes	Shell surface	Medium
* *E. zelandica*	?	—	Very faint traces	
* *Isanda coronata*	S	Yes	Shell surface	Medium
* *I. pudibunda*	S	Yes	Shell surface	Medium
* *I. rhodomphala*	S	Yes	Umbilicus	Strong
* *Monilia calyculus*	S	No	Umbilicus and columellar lip	Medium
* *M. philippii*	S	Yes	Umbilicus	Medium
* *M. lifuana*	S	No	General	Trace
Thalotia conica	?	—	Very faint traces	
Elenchus bellulus	?	—	Very faint traces	
E. irisodontes	?	—	Very faint traces	
Umbonium vestiarium	S	No	General	Strong
* *U. giganteum*	S	Yes	Lip and umbilicus callus	Strong
U. suturale	S	Yes	Umbilicus callus	Strong
* *U. javanicum*	S	?	Bands, sutures, callus	Medium
* *U. conicum*	S	No	General	Strong
U. costatum	S	Partial	Lip and callus	Strong
U. australe	S	Yes	Bands, lip and callus	Strong
U. moniliferum	S	Yes	Callus only	Strong
Livona pica	S	Yes	Black areas	Strong
Gibbula magus	S	Yes	Red areas	Weak
G. cineraria	S	No	Apical callus	Strong
Monodonta colubrinum	S	—	General	Traces
M. confusum	S	—	Patch near denticle	Weak

Table 1 (*cont.*).

	Emission tint	Conformity with pigment pattern	Site	Intensity
* *Angaria* spp.	S	No	General	Strong
Leptothyra sanguinea	S	Yes	Red areas	Medium
Lithopoma americana	S	No	Patch on columellar lip	Very strong
Astraea triumphans	S	Yes	Umbilicus callus	Strong
Tricolia pullus	S	Yes	Individuals	Medium
T. elongata	E	Yes	Yellow individuals	Weak
Theodoxus fluviatilis	S	Yes	Individuals	Traces
T. prevostianus	S	No	Patchy	Traces
Neritodryas dubius	S	Yes	Pink bands, red form only	Strong
* *Neritina communis*	S	Yes	Pink bands	Strong
Torinia variegata	S	Yes	Red areas	Medium
* *Velutina laevigata*	E	—	Orifice, young shells	Weak
V. zonata	E	—	—	Traces
Erato vitellina	S	Yes	General	Strong
E. laevis	S	No	Lip only	Strong
E. lachryma	S	Yes	Bands	Strong
E. columbella	S	Yes	General	Strong
E. cimaculata	S	Yes	Spots	Strong
E. sulcifera	S	Yes	General	Medium
* *Trivia ovulata*	S	No	General	Weak
T. arctica	S	No	General	Strong
T. monacha	S	No	General	Strong
T. quadripunctata	S	No	General	Medium
T. pediculus	S	Yes	Not dark areas	Medium
T. oryza	S	—	—	Traces
* *T. merces*	S	Yes	Dark areas only	Medium
Cypraea cinerea	S	No	General, esp. extremities of mouth	Strong
* *C. mappa*	S	No	General	Very strong
* *C. subviridis*	S	No	General	Weak
* *C. pulchra*	S	No	General	Weak
* *C. isabella*	E	—	—	Trace
Marginella ornata	S	No	General in young shells, later red areas	Medium
* *Hydatina physis*	E	No	Transverse growth lines	Weak
Bulla ampulla	S	Yes	Pink and purple areas	Medium
B. adansonii	S	Yes	Pink and purple areas	Medium
* *B. quoyi*	S	Yes	Pink and purple areas	Medium
Actaeon tornatilis	S	Yes	Pink bands	Medium
Haminea vesicula	E	Yes	General	Strong
* *Aplustrum amplustre*	E, S	Yes	Lilac and pink bands	Strong
Umbraculum mediterraneum	S	Yes	Visceral stain	Traces

* Identification based on British Museum labels.

Table 2. *Other colours of emission in Gastropods*

	Emission tint	Site
Proserpina nitida	Yellow	General
Helix aspersa	Yellow	Areas contaminated with foot mucus
* *Alcadia rhodostoma*	Yellow	Lip and operculum
Cypraea tigris	Orange	Shell of orange form
Patella depressa	Orange	Visceral stain
Murex regius	Orange pink	Lip
Fasciolaria tulipa	Orange pink	Lip
Papuina boivini	Orange	Lip
Subulina octona	White	Contained eggs

* Identification based on British Museum labels.

Land and fresh-water gastropods

With the exception of *Theodoxus*, we detected no porphyrin in the shells of any fresh-water forms. Long series of land operculates and pulmonates of all groups were equally negative. The observation of porphyrin in the dermis of slugs, while telling against any argument based on photosensitizing effects, has no counterpart in our study of shells.

Pink or orange-pink fluorescence of a very low order, not extractable by acid, was observed in a number of land shells. The species concerned were

all white, xerophilic or desert forms, and mostly in a weathered or sunbaked state, a control series of fresh material being negative. The pigmentation was patchy, confined in some cases to the brown bands, and in others to areas which had been in contact with the ground or with moisture. The species concerned were: *Rumina decollata*, *Ena detrita*, *E. exilis*, *Strophia uva*, *Geomitra nitidiuscula*, *Otala lactea*, *Eremina desertorum*.

One species (*Bulimulus reentsi*) showed intenser orange-pink fluorescence, not typical of porphyrin, and most marked in the youngest parts of the shell. No material was available for extraction.

We regard this pink fluorescence as of non-porphyrin origin, due possibly to the existence of fluorescent, crystalline forms of calcium carbonate in old shells.

Pelecypoda

Beside *Pteria* (*Pinctada*), whose fluorescence was first noted by Fischer & Jordan (1930), porphyrins occur in some Anomiidae (*Placuna* and *Enigmonia*), in *Pinna*, and in *Malleus*, *Isognomon*, *Vulsella*. The only higher bivalves in which we found any trace of red fluorescence were *Venus* (*Clausinella*) *fasciata*, *Gafrarium divaricatum* and *Sunetta solandrei*. Apart from these three species, the porphyrin-containing bivalves have a superficially similar shell structure and a tendency to produce rays of brownish or purplish pigment which, in many individuals, is non-fluorescent. *Malleus vulgaris*, which produces large amounts of easily extractable acid-soluble violet pigment, shows streaks of porphyrin in the region of the hinge. The extract is not fluorescent in solution. *M. regula* was found to contain small amounts of extractable porphyrin with large quantities of other, chromatographically distinct, pigment.

Anatomical distribution

Shell porphyrin fluorescence is of four main types: (i) Generally diffused; (ii) sharply coincident with the visible pigmentary pattern; (iii) localized in non-pigmented or faintly pigmented areas which are constant for the species; (iv) confined to one or more bands in a visible pigmentary pattern.

Forms showing general diffusion include some *Trivia*, and several *Fissurella*. Pigmentation of the second type is of great interest, since porphyrin may be absent from the pigmented areas of two otherwise identical shells, and its intensity bears no relation to that of visible pigment. The third type of distribution suggests the association of porphyrin deposition with a particular organ, the visceral hump of *Umbraculum*, or the columellar mantle in some *Trochi*. Univalves frequently show localization, if any, in the region of the mouth, porphyrin being confined to the umbilical callus in some *Umbonium*, to a pink band in others: to a patch on the lip in *Lithopoma*, to the callus occupying the site of the old protoconch in *Gibbula cineraria*, and, as already mentioned, to the chlamydogenous areas of many Cypraeids. Coincidence with a band, usually pink, is most marked in *Aplustrum* and some species of *Umbonium* and *Neritina*. In *Pinna*, pigment is often confined to a small area inside the valve and near the middle.

Coincident pigment. The visible pigments of porphyrin-containing forms show an emphatic preponderance of purple, red, and brown. In pigmentary patterns of type (ii) above, the coincident pigment may be black (*Livona pica*), scarlet (*Trochus niloticus*, *Clanculus puniceus*), pink (*Aplustrum amplustre*) or brown (*Pteria*).

In some cases there is definite correlation between the brightness of fluorescence and the depth of colour of the seasonal pigment varices, but closely related species, or individuals of the same species, of identical colour, show wide variation in the porphyrin content, and in almost all instances the porphyrin fraction is chromatographically separable from the main coloured material. In *Trivia pediculus* the whole dorsum except the brown spots fluoresces: in the closely similar *T. merces* the position is reversed.

Site. In no instance have we found porphyrin in nacreous shell, though it may occur in callus laid down by the somatic parts of the mantle. In many forms it is sharply confined to the surface layer of the shell, next to the periostracum, where this is present. In *Placuna*, which has a laminar structure rather like mica, the pigment is sandwiched between the layers of shell.

Relation to haemoglobins

Molluscan haemoglobins may be circulatory or confined to the radular muscle. We could not establish any relation between the occurrence of pyrrolic respiratory pigments and the presence of shell porphyrin. Forms such as *Arca* and *Planorbis*, which are known to have red blood, are devoid of shell porphyrin.

Individual variation

Of twenty specimens of *Venus* (*Clausinella*) *fasciata*, only five showed fluorescence, and in two of these intense fluorescence was noted. One of the brightest specimens belonged to the permanganate-coloured variant, though comparable purple variants of *Dosinia* and several other Veneridae contained no detectable porphyrin. Fluorescence in the other two venerids was likewise confined to pink or purplish specimens.

In *Trivia monacha*, *Cypraea isabella*, *C. mappa*, and *C. cinerea*, non-fluorescent individuals are not

uncommon, and intermediate forms showing porphyrin only round the mouth and in the terminal calli also occur.

Fluorescence is often most marked in small examples of a species, as though dilution by shell matter were taking place.

Individual variation in most forms is as marked as variation in the visible intensity of pigmentation, and it is, therefore, impossible to exclude porphyrin formation without examining long series. In the light of this finding, a number of our negative results may well be revised by more extensive studies.

Relation to body colour

Since shell porphyrins are almost certainly a product of gradual accumulation, high concentrations of fluorescent material are not necessarily to be expected in the mantle. This important study could not be pursued in the most suitable forms, such as *Pinctada*, but in the British *Trivia* fluoroscopy of the living animal showed no evidence of large tissue concentrations of porphyrins. The siphon and mantle borders exhibit slight reddish fluorescence, but the main pigment, an orange, acetone-soluble material, proved to be a complex mixture of carotenoids, giving seven zones on chromatography, while extracts of the ground animal in 0·2N-hydrochloric acid showed no red fluorescence whatever if free from shell fragments. The bright orange faeces of *Trivia* are also without fluorescence.

Relation to calcium metabolism

The irregularity with which porphyrins occur in shells, and the high concentrations which exist in many thin, poorly calcified forms, do not suggest that they play any major part in shell deposition, although they are known to appear in the dart sac of *Helicids* during the process of calcification (Kühnelt, personal communication). The extremely high concentrations present in many forms favour the idea that porphyrins are secreted with the shell as a means of disposal.

Relation to molluscan taxonomy

Our findings for distribution agree well with the anatomical classification of Thiele (1929, 1931). Porphyrins, although absent from recent species of *Pleurotomaria*, and replaced by other pigments in *Haliotis*, appear to be widespread among the less specialized Archaeogastropoda. Apart from outlying forms such as *Marginella* and the few Veneridae, the other porphyrin-depositing groups are almost equally clearcut. In *Cypraea*, for instance, the subgenus *Luria*, created on anatomical grounds, is a self-contained focus of porphyrin deposition. Even the superficial similarity of appearance between *Erato* and *Marginella*, unrelated forms, appears to be accompanied by a convergence of pigment metabolism. The comparative biochemistry of such resemblances should certainly be studied further.

Direct spectroscopy of shells

Porphyrin spectra were obtained in a number of bivalves and in *Umbrella* by direct transillumination of the shell. Fischer & Jordan (1930) had examined '*Pteria radiata*' by this method, and found bands at

	6321–6168	5882–5650	5508–5408	5172–4979 A.
max.	6244	5766	5458	5075

Our own readings for porphyrin-containing and control species were as follows (Table 3):

Table 3. *Absorption spectra of shells by direct transillumination*

	Abs. maxima (A.)				Fluorescence
Umbraculum mediterraneum (edge)		Nil			Nil
U. mediterraneum (visceral stain)	6220	5900	5465	5120	Yes
* *Pteria chinensis*	6210	5804	5480	5010	Yes
* *P. castanea*	6200	5955	5420	5095	Yes
* *P. rufa*	—	—	—	5010	Yes
* *P. hirundo*	—	—	—	5015	Yes
* *P. electrina*	—	—	—	5035	Yes
* *Enigmonia enigmatica*	—	—	—	5000	Yes
* *Placuna sella*	—	—	—	5000	Yes
Venus fasciata (pink)	—	—	—	5070	Yes
* *Umbraculum cumingii* (edge)	—	—	—	5010	Nil
U. cumingii (visceral stain)	—	5975	5490	5010	Yes
Chlamys tincta (yellow form)	6900†	—	—	5000	Nil
C. tincta (orange-red form)	7100†	—	5140	4950	Nil
C. tincta (red form)	6900†	—	5045	5000	Nil
C. tincta (orange form)	7000†	—	5042	5000	Nil

Probable error in estimating maxima of very low intensity ±20 A.
* Identification based on British Museum labels.
† Limit of transmission in the red.

The scope of the present work is limited to the general distribution of red fluorescence in shells. Of the spectral bands detected some at least are due to concomitant pigments of non-porphyrin character which have been separated chromatographically. Details of these, and of the chemistry of isolated shell porphyrins, will be published in forthcoming communications.

SUMMARY

1. The distribution of red fluorescence in molluscan shells is described. It is absent from land and fresh-water forms, and commonest in the Archaeogastropoda, appearing also in the Lamellariacea, certain Cypraeidae, the tectibranch opisthobranchs, *Umbraculum*, the pearl oysters, and sporadically elsewhere. The limits of distribution coincide closely with the existing anatomical nomenclature.

2. Porphyrins in shells are commonly associated with a wide variety of other acid-soluble pigments.

3. Both these and the porphyrins are at present under fuller investigation, of which details will appear separately.

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Characterization of Sugar Components of Proteins

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Carbohydrates form an integral part of many biologically active complexes and the problem of their estimation in the presence of protein is common. The classical methods are inapplicable to the small amounts generally involved, and numerous colour reagents have been used for the detection of various sugars. Their number and the modifications of the few reactions which have been developed on quantitative lines reflect the existing state of dissatisfaction. A critical investigation of the problem seemed desirable.

The ideal of a specific visible absorption assay for each sugar has not been realized, nor has it been possible to find a reagent giving equal colour intensities for equal quantities of sugar, irrespective of the configuration, so that current methods necessitate qualitative characterization before quantitative estimations can be attempted.

Colour reagents for sugars are either phenols or polyphenols with the substituents in the meta position (thymol, phloroglucinol, α- and β-naphthol, resorcinol, orcinol), or nitrogenous compounds, such as indole, skatole, diphenylamine, urea or guanidine. A range of thirty-two compounds of these two types was examined under standard conditions in concentrated sulphuric acid solution for colour formation with four representative hexoses, namely mannose, galactose, glucose and fructose. In every case colours were obtained, and distinction between mannose and galactose on the one hand, and glucose and fructose on the other hand, was possible. Mannose and galactose, however, showed similar absorption spectra. Fructose gave colour reactions more intensely and more quickly than the aldo sugars. The absorptions, especially in the case of the aldo sugars, were not intense enough to warrant a quantitative investi-