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A Note on the Disturbance of the Haemoglobin Metabolism of the Rat by Sulphanilamide

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Certain disturbances of haemoglobin metabolism were among the side reactions observed after the introduction of sulphonamide therapy. Animal experiments clearly demonstrated that continued dosage produced an anaemia. In human subjects cyanosis was common, due mainly to methaemoglobinaemia and occasionally to sulphaemoglobinemia. Drug rashes were also observed in a proportion of cases, and, while not all of the rashes were associated with light sensitivity, the excretion of urinary porphyrin after dosage was found to be increased in a number of cases (cf. Rimington & Hemmings, 1938). The excretion of porphyrin in the urine of the rat was then found to be increased many times by dosage with sulphonamides (Wien, 1938; Rimington & Hemmings, 1938, 1939). Other aromatic amino compounds were found to produce a porphyrinuria. Rimington & Hemmings (1939) observed a correlation between the severity of the porphyrinuria and the presence of methaemoglobin in the blood of their animals. Since the porphyrin was found to be coproporphyrin type III, differing only from the protoporphyrin IX found in haemoglobin

in having propionic acid side chains in place of vinyl side chains, they put forward the tentative hypothesis that the coproporphyrin was derived from the increased haemoglobin breakdown under these conditions. A similar view was entertained by Brownlee (1939) as a result of his investigation of the effects following the administration of antipyretics to rats. The appearance of coproporphyrin would signify, according to this hypothesis, a departure from the normal pathway of haemoglobin catabolism.

The present work was begun in order to see if this porphyrinuric action of the sulphonamides in rats could be mitigated by the administration at the same time of substances, such as ascorbic acid, hydrochloric acid, etc., which had been claimed to diminish the severity of the side reactions following sulphonamide therapy. In addition, it was felt desirable to investigate quantitatively the bile-pigment excretion during dosage with the drug so that an estimate might be made of the importance of the normal pathway of haemoglobin catabolism under these conditions. Rimington & Hemmings (1938) had indeed commented on the large amounts of urobilin which they observed in the course of their analyses of faecal porphyrins.

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It was not found possible, however, in our experiments, to repeat the basic observations of Wien (1938) and of Rimington & Hemmings (1938), i.e. increased excretion of coproporphyrin after dosage with the drug, and in consequence the immediate clinical importance of the programme was greatly diminished. Transfer of the author to other work has prevented the carrying out of further experiments to elucidate the cause of the disagreement. However, attention has not been drawn to the problem since the completion of the experimental work outlined here (1942) and since additional disturbances of pigment metabolism were noted, the data are presented here in the hope that further interest may be aroused. The positive findings of several individual groups of workers would suggest that the cause of our negative findings might be due to some factor, such as the dietary, and the elucidation of this point would be of no little interest.

EXPERIMENTAL

Adult white rats, 200–300 g. in weight, were used.

Diet. Two diets were used: *A*, ground wheat meal 86%, dried whole milk 7%, meat meal 3.5%, bone meal 3.5%; *B*, wheat meal 60%, dried whole milk 10%, meat meal 25%, bone meal 5%. Diet *A* was the stock diet of the rat colony. The animals remained healthy on this diet and reproduced normally. During dosage with sulphanilamide, however, a higher mortality was observed than other workers had reported. Since Smith, Lillie & Sholman (1941) had observed that rats on a high-protein diet showed no decline in haemoglobin when dosed with sulphanilamide, and exhibited a lower mortality than when on a low-protein diet, an experiment was carried out with diet *B*.

During an experiment the animals were given water *ad lib.*, and were given free access to the diet for 1 hr./day. They remained healthy under these conditions when not dosed with the drug.

Collection of samples. Twelve individual metabolism cages were used. They were supported on waxed aluminium funnels and delivered the urine and faeces on to bulbs, sitting in the necks of 100 ml. flasks. The urine was collected in the flask which contained a few drops of toluene, the faeces in a tin surrounding the flask. Mechanical losses of urine amounted to no more than 5% of the total volume. Analyses were carried out on 3-day collections of the urine and faeces.

Administration of drug. Solutions or aqueous suspensions of the drugs used were delivered directly into the oesophagus of the animals with a blunt pipette after their daily feed.

Analytical methods

Urine coproporphyrin. Bulbs and flasks were inspected daily so that urine contaminated with fragments of faeces might be discarded; the samples were then filtered through glass wool and the coproporphyrin taken into ether by adding one third volume of acetic acid and shaking with three portions of solvent. The ether was washed once with a solution of sodium acetate, five times with distilled water and the porphyrin extracted with three portions of approx. 0.1*N*-HCl. The concentration of porphyrin in the extract

was determined by matching its fluorescence in filtered ultraviolet light with the nearest of a series of dilutions of mesoporphyrin IX in approx. 0.14*N*-HCl. A red filter, transmitting beyond 630 mμ, excluded the whitish fluorescence of an interfering substance not removed by the preliminary purification. A spectrophotometric check showed that the error involved by using a different porphyrin for comparison could be neglected.

Faecal porphyrin. Ground faeces were left covered with glacial acetic acid for 30 min. They were then thrice extracted with an equal volume of ether. The acid-ether extract was then shaken with about 15 ml. sodium acetate and with successive portions of water until only traces of urobilin were present in the extract. The sodium acetate and the water washings were retained for the urobilin estimation. Both copro- and proto-porphyrin were then removed from the washed ether by three extractions with approx. 1.4*N*-HCl. The combined extracts were filtered and the concentration of the porphyrin determined by measurement at 552 mμ in a Hilger-Nutting Barfit spectrophotometer.

The total porphyrin was measured in this way because it was not found possible to get a good separation of the two porphyrins by acid fractionation of the crude extract of faeces. The measurement is thus a rather crude index of changes in the excretion of coproporphyrin. At the end of a given experimental period the pooled extracts were further purified and the copro- and proto-porphyrins extracted and determined separately.

Fig. 1. (a), tetrahydromesobilene-*b*; (b), mesobilene-*b*; *E*=ethyl, *M*=methyl, *P*=propionic acid.

Urobilins in faeces. Two related members of the urobilin class are generally obtained from both faeces and urine. Their structures are illustrated in Fig. 1. In the present paper structure I (a) will be termed tetrahydromesobilene-*b*, and structure I (b), mesobilene-*b*.* Repeated attempts were made to estimate the total amount of both these urobilins by reduction to their leuco compounds—the corresponding urobilinogens or mesobilanes—followed by

* This step represents a simplification of bile pigment nomenclature (cf. Siedel, 1939). It is considered necessary because of the confused state of the nomenclature of the bile pigments and particularly of the urobilins, where tetrahydromesobilene-*b* may be called stercobilin or urobilin, and mesobilene-*b*, urobilin or urobilin IXα. The problem will be adequately discussed by Lemberg in a forthcoming monograph.

coupling with Ehrlich's reagent. These attempts failed. The pigments were, therefore, determined in the spectrophotometer as their hydrochlorides; this derivative being formed by acidifying the washings of the ether extract of the faeces (*vide supra*) with HCl. The specific extinction coefficient $E_{1\text{ cm}}^{0.1\%}$ at 492 m μ . was found to be 147 by measurement of a crystalline sample of the hydrochloride of tetrahydromesobilene-*b*. The extinction coefficient of mesobilene-*b* was assumed to be equal to that of the tetrahydro compound.

Mesobilene-b. While tetrahydromesobilene-*b* is stable to FeCl_3 oxidation, mesobilene-*b* is oxidized to a biliviolin. This reaction, which was used in a semi-quantitative fashion by Lemberg, Lockwood & Wyndham (1938), is carried out in the following way. The aqueous solution of the urobilins remaining from the previous estimation is extracted with 10–15 ml. of chloroform. The extract is then evaporated to dryness on a water bath and the residue taken up in 5 ml. of methanol. A sample is suitably diluted with methanol, and its extinction coefficient measured at 492 m μ ., thus giving a measure of the tetrahydromesobilene-*b* plus mesobilene-*b*. To 4.5 ml. of the remaining methanol solution are added 0.5 ml. of 20% (w/v) FeCl_3 in HCl. The solution is refluxed in a test tube for 15 min., cooled, the pigments taken into ether with sodium acetate and after thorough washing to remove as much unchanged tetrahydromesobilene-*b* as possible, the violins are extracted with approx. 2.8N-HCl. The acid extract is read in the spectrophotometer at 492 and 560 m μ . The violin absorption is negligible at the former wave length, 492 m μ ., the absorption here being that of traces of tetrahydromesobilene-*b*; the absorption of the latter compound at 560 m μ . is 0.07 times that at 492 m μ .; this value is subtracted from that found at 560 m μ ., thus enabling the true violin absorption at this wave length to be obtained. Not enough of the violin was obtained to enable a determination of its extinction coefficient but Lemberg, Lockwood & Legge (1941) have found the specific extinction coefficient of a strongly basic violin to be the same as that of biliverdin, $E_{1\text{ cm}}^{0.1\%} = 46$. This value was taken for the violin obtained from the mesobilene-*b*. A sample of mesobilene-*b* prepared 5 years previously by Lemberg *et al.* (1938) from crystalline mesobilene (mesobilinogen) gave a mixture of biliviolin and biliverdin on oxidation, and being analyzed by the above procedure gave 120% together of these two bile pigments calculated on the original mesobilene-*b*. The compound in faeces gave no biliverdin on oxidation. This difference in behaviour prevented a more accurate check on the procedure.

Abnormal blood pigments. The blood was diluted, saturated with coal gas and examined with a hand spectroscope. A band in the 620–630 m μ . region of the spectrum could denote methaemoglobin, sulphaemoglobin or choleglobin. A weakening or disappearance of the band on reduction with hyposulphite ($\text{Na}_2\text{S}_4\text{O}_6$) indicated the presence of methaemoglobin. Alkali was now added, a weakening or disappearance of the band indicated the presence of sulphaemoglobin. The remaining band is that of CO-cholehaemochromogen (Lemberg, Legge & Lockwood, 1941).

RESULTS

The excretion of the porphyrins and urobilins was measured over periods up to 20 days in about 100 rats. The data may best be summarized by reference

to the results obtained in an experiment in which 12 rats were fed on diet *B* (group *J*). They were kept in metabolism cages for 12 days before dosing was commenced with 1 g./kg. of sulphanilamide daily. Analyses were commenced 6 days before the dosing commenced and continued for a further 18 days. The mean values for the 3-day excretion of urinary coproporphyrin, mixed faecal porphyrins, mixed

Fig. 2. Mean excretion/3-day rat period of: *A*, faecal urobilins; *B*, faecal porphyrins; *C*, faecal mesobilene-*b*; *D*, urine coproporphyrin. The vertical dotted line indicates commencement of dosage with sulphanilamide. The vertical unbroken lines indicate range of values of urinary coproporphyrin.

faecal urobilins and faecal mesobilene-*b* are plotted in Fig. 2 on a logarithmic scale so that the quantitative relations between these substances may be more easily appreciated. The range of values found for the excretion of the urinary coproporphyrin within the group at each period is indicated by the vertical lines on the appropriate graph in Fig. 2. The range of values found for the excretion of each of the other derivatives is given separately in Table 1. The results from animals which did not survive a particular 3-day period were excluded from computation of the mean.

Table 1. *Excretion of faecal pigments by group of rats dosed sulphanilamide (1 g./kg.)*

Days ...	Control period		Period of sulphanilamide dosing					
	3	6	3	6	9	12	15	18
Porphyrins:								
Range ($\mu\text{g./3 days}$)	170-420	140-260	170-400	160-370	130-430	120-570	140-330	160-310
Urobilins:								
Range ($\mu\text{g./3 days}$)	430-910	320-660	190-610	190-560	410-1880	530-1890	360-1020	350-530
Mesobilene-b:								
Range ($\mu\text{g./3 days}$)	14-28	7-24	7-38	12-160	22-160	57-400	78-610	33-190
Surviving rats	12	12	12	9	9	8	8	7

Urinary coproporphyrin

Throughout the period of the experiment, the mean excretion of this derivative remained about constant. This result was typical of those obtained in the other experiments. The control excretion was the same as that found in rats on diet A. The individual records of all the rats show only two cases, both on diet A, which are not in accord with the above statement. Rat 69 excreted 3.7 and 6.4 $\mu\text{g.}$ coproporphyrin during two control periods and 2.4, 5.3, 13.6 and 17 $\mu\text{g.}$ coproporphyrin during four periods in which it was dosed with 1 g./kg. sulphanilamide. The animal did not survive a fifth period of dosing and was the only one in which the increase in excretion reported by other workers was found. Another animal, number 71, showed an unusually high excretion throughout the experiment. The coproporphyrin excretion for the control periods was found to be 11.0 and 10.0 $\mu\text{g.}$, and on five succeeding 3-day periods of dosage 7.0, 9.9, 12.5, 19.4 and 10.6 $\mu\text{g.}$ respectively. At this point the experiment was discontinued, the rat surviving. No significance is attached to the fact that both these animals were male or were found to be in the same group. Inspection of individual records failed to reveal any change in the excretion of the urinary coproporphyrin in the periods prior to the death of the rat, nor did scatter diagrams show any correlation between excretion of total faecal porphyrins and excretion of urinary coproporphyrin, or between the number of reticulocytes/cu.mm. and the excretion of urinary coproporphyrin.

Insufficient pooled urinary porphyrins were obtained at the end of the experiments to permit identification by confirmed methods of the proportion of types I and III present. Some preliminary experiments by the method of Watson & Schwartz (1940) (elution by 35% aqueous acetone and by 100% acetone of the methyl esters of coproporphyrins I and III respectively from Brockmann's alumina) indicated that over 90% of the porphyrin in the urine of both normal and dosed animals was type III.

Faecal porphyrins

In none of the experiments were the faecal porphyrins observed to increase in amount following dosage. The general tendency following dosage was, indeed, a decrease from 300-400 to 200-300 $\mu\text{g./3-day}$ period in rats on diet A. Although this decrease was not observed in the rats on diet B, the variation in excretion between different rats, as evidenced by the data in Table 1, was typical of the other experiments. The porphyrins from successive 3-day periods from each rat were retained, and in several experiments were further purified so that the proportion of coproporphyrin in the mixture might be determined. This was found to vary, apparently at random, between 10 and 30% of the total porphyrin. Neither the type of diet nor the dosage with sulphanilamide had a predictable effect.

Total faecal urobilins

The increase in the mean excretion of urobilins from between 500 and 600 $\mu\text{g.}$ to between 700 and 1000 $\mu\text{g./period}$, after 9-12 days' dosage with sulphanilamide, was observed in all the experiments. The highest excretion observed, 1890 $\mu\text{g.}$, represented a 2.5-fold increase, while between one-third and one quarter of the animals showed a twofold increase on the highest pre-dosage excretion. The values in Table 1 indicate the considerable variation in the response. Some animals, indeed, failed to show any significant increase in excretion even when their erythrocytes dropped to half their normal level in 15 days.

There was no correlation between the doubling of the pre-dosage excretion of the urobilins and the survival or death of the animal. After the dosage with the drug had continued for 12-15 days some increase was observed in the concentration of the urobilins in the urine. This was not measured quantitatively.

Faecal mesobilene-b

After the experiments had been in progress for some time, it was noticed that the colour of the dilute sodium acetate solution of the urobilins

changed in hue from a rich yellow to a pinkish yellow. This was shown to be due to the presence of increased amounts of mesobilene-*b*. At first the amount of this pigment present was estimated qualitatively by the intensity of the band at 634 m μ . seen after the addition of copper acetate to a methanol solution of the urobilins, the Cu⁺⁺ oxidizing the mesobilene-*b* to a violin and forming its copper salt while leaving the tetrahydromesobilene-*b* unchanged. In later experiments the quantitative procedure outlined above was used.

The increase in the mean excretion of mesobilene-*b* seen in Fig. 2 was also seen in the other group of rats whose mesobilene-*b* excretion was followed throughout dosage. As with the excretion of total urobilins, the response was observed to be variable. Of the thirteen animals who survived 15 days' dosage with the drug, only three failed to show less than a three-fold increase in mesobilene-*b*. The greater part of the increase in the excretion of the total urobilins is, however, due to the increase of tetrahydromesobilene-*b*. Thus, rat 89 showed an increase in total urobilin excretion of 1400 μ g. between the sixth and ninth days of dosage, of which only 150 μ g. is mesobilene-*b*.

The mesobilene-*b* excretion of individual rats appeared unrelated to their survival or death during the experiment. Examination of the pre-dosage levels of mesobilene-*b* of rats on diets *A* and *B* indicated, however, that the excretion was significantly less on diet *B* (Student's *t* test, *P* less than 0.001).

In addition to the increased mesobilene-*b*, another abnormal pigment was observed. The porphyrins extracted from the washed ether extract of faeces obtained from animals, at the same time as the mesobilene-*b* excretion had increased, were observed to be bluer in colour than extracts obtained at an earlier period. This was shown to be due to the presence of a strongly basic biliviolin. Insufficient was obtained to show whether it was identical with that obtained by ferric chloride oxidation of mesobilene-*b*, but this appears probable. Rimington & Hemmings (1938) also found blue pigments in the acid washings of the ether extract of faeces from rats dosed with sulphanilamide.

General observations

Haemoglobinuria was observed on 8/465 rat days during control periods and on 19/662 rat days during dosage. The difference is not significant and it is improbable, therefore, that the phenomenon is caused by the drug.

A green pigment was occasionally observed in voided urine (cf. Maxwell & Whipple, 1929). This is probably due to the breakdown of oxyhaemoglobin to choleglobin since the characteristic band of CO cholehaemochromogen could be observed after appropriate treatment. In addition, flocks of bili-

verdin hydrochloride (showing the characteristic band at 670 m μ .) were obtained in the 0.14N-hydrochloric acid extract of the ether solution obtained in the course of routine analysis of the above urines for coproporphyrin.

When dosage with sulphanilamide was commenced, the increase in volume of urine observed by Rimington (1939) was not found (cf. Brownlee, 1939).

The amount of faeces generally showed an initial decrease when dosage commenced, rising after the first 2 or 3 days to the usual level.

There was generally a slight initial weight loss from which the animals recovered. Group J showed a drop in mean weight from 245 to 216 g. in the 10 days spent in cages prior to commencement of dosing. During dosing the mean weight of the survivors fluctuated between 200 and 220 g.

In all the experiments the rats became cyanosed. The main abnormal pigment proved on examination to be sulphaemoglobin, with smaller amounts of methaemoglobin and choleglobin present. In this respect also the results differ from those of Rimington (1939), who only reported methaemoglobin to be present.

The survivors of the group on diet *B* showed an average drop in erythrocytes from 9.2 to 5.4 millions/cu.mm. after 12 days' dosage, while the reticulocyte count rose at the same time from 1.8 to 7%. A similar result was found in another group.

The mortality observed in these experiments was greater than that observed by other workers. In the group on diet *B* only seven twelfths of the animals survived 18 days' dosage at 1 g./kg. level. The mortality among the rats fed on diet *A* was greater. The only obvious evidence of dietary abnormality observed in the rats on either diet was an occasional discharge of porphyrin-containing fluid from the glands of Harder, the so-called 'bloody whiskers'.

DISCUSSION

In these experiments the sulphanilamide produced a severe anaemia in the rats. No increase was observed in the urinary excretion of coproporphyrin or in the level of the faecal porphyrins. The increased content of urobilins in the faeces strongly suggests that the accelerated haemoglobin breakdown proceeds by a normal route forming bile pigments and not porphyrins. Certain animals, although anaemic, did not show significant changes in the total amount of urobilins excreted. It is known, however, that, owing to their destruction in the gut, the excretion of urobilins is an unsatisfactory index of the amount of haemoglobin catabolized. If it is assumed that the life of the red cell in the rat is the same as that found in other mammalian species, about 120 days (dog, Hawkins & Whipple, 1938; man, Shemin & Rittenberg, 1945; Jope, 1946), the normal excretion of the

urobilins in rat faeces represents a recovery of about 20 % of the prosthetic group actually destroyed.

The conclusion drawn from the association between the increased destruction of haemoglobin and the increased output of the urobilins might, perhaps, be queried, on the grounds that the pigments are found in increased amount because there is less destruction in the gut. There is, indeed, some evidence (*vide infra*) of altered bacterial activity in the gut, but none that this leads to a diminution of the overall destruction of the urobilins.

In the light of the results reported in this paper, the hypothesis put forward by Rimington (1940), as to the origin of the increased urinary coproporphyrin, can no longer be supported. The results found in the present series of experiments are not the only ones suggesting that the problem is more complicated. While Wien (1938) found an increased excretion of coproporphyrin after sulphanilamide, he failed to find any increase after 15 days' dosage with 3 g./kg. sulphapyridine, although Rimington (1940) found a fivefold increase in coproporphyrin excretion under similar conditions. Prof. Rimington has kindly informed me that he has heard from Dr M. Guggenheim, Hoffmann-la Roche Ltd, Basle, of another instance, where, under slightly different conditions, no increase in the excretion of coproporphyrin was noticed in rats after sulphanilamide treatment.

While it is possible (cf. Figge, Strong, Strong & Shanbrom, 1942; Bittner & Watson, 1945) that genetic differences may lie at the basis of the discrepancy between these results and those of Wien, of Rimington and of Brownlee, it seems more likely that they are of nutritional origin. The fact that substances with negligible bacteriostatic properties, such as aspirin, phenacetin, phenazone (Brownlee, 1939) or aniline (Rimington & Hemmings, 1939), produce the porphyrinuria make it unlikely that the explanation is to be sought in the diminished bacterial synthesis of vitamins in the intestine. It is, therefore, probable that exogenous factors are involved. Mortality under dosage is perhaps the most important biological criterion of the physiological condition of the animals. Since the mortality in the present experiments was greater than that reported by other workers it seems most likely that factors present in other diets were absent in that used in the present series. On this view the porphyrinuria may be simply part of the normal erythropoietic response, perhaps slightly deranged by the drugs, but not of any great pathological importance for the animals. It should be noted, however, that in the present experiments a reticulocyte response was certainly present.

On an alternative view, the diet on which a porphyrinuria is found after drug treatment is not as complete as one on which no such response occurs.

This seems less probable than the former hypothesis, but cannot at present be entirely excluded. Ellinger, Edgar & Lucas (1935) have reported a porphyrinuria in rats on certain diets, while the various deficiencies producing porphyrin staining of the fur and whiskers (cf. Daft & Sebrell, 1945), have yet to be thoroughly investigated from the point of view of porphyrin metabolism. Raoul & Marnay (1945*a, b*) failed to find an increase in urinary coproporphyrin when rats on a diet deficient in pyridoxine or pantothenic acid showed a cutaneous excretion of protoporphyrin.

Finally, comment must be made on the increased excretion of mesobilene-*b*. The only previous observation which related to the amounts of mesobilene-*b* and tetrahydromesobilene-*b* excreted were those of Lemberg *et al.* (1938). They examined urines from patients with various complaints, but observed no association between increased excretion of urobilins in the urine and the proportion of mesobilene-*b* to tetrahydromesobilene-*b*. Nor was there a significant association between increased mesobilene-*b* excretion and liver damage. The increase in mesobilene-*b* in the present series of investigations provides, therefore, a further approach to the complex physiology of the urobilins. Baumgärtel (1943*a-d*), in a series of papers available to the author only in abstract, has reported experiments which indicate that bacteria cannot reduce biliverdin, and that only after this compound is reduced to bilirubin by the liver (Lemberg & Wyndham, 1936) does bacterial catalysis of further hydrogen transfer commence. The complete reduction of the conjugated double bond system of the bilirubin is reported by Baumgärtel to follow a series of steps in which (1) the $\alpha:\beta$ double bonds of the terminal pyrroles are reduced, (2) the hydrogens shift from this position to the α and c methene bridges, forming mesobilane, the precursor of mesobilene-*b*, (3) the $\alpha:\beta$ double bonds of the terminal pyrroles are again reduced forming tetrahydromesobilane, the precursor of tetrahydromesobilene-*b*. On this hypothesis the increased amounts of mesobilene-*b* found after dosage with sulphanilamide result from a partial inhibition of step (3). It is uncertain whether such an inhibition takes place at the enzyme level, or whether it is due to alterations in the composition of the bacterial population. It would be useful to reinvestigate the varieties of bile pigments excreted following the administration of other sulpha drugs (cf. Greenblatt & Greenblatt, 1945).

SUMMARY

1. After treatment with sulphanilamide, rats were observed to show increased excretion of urobilins. The reported increase in urinary excretion of coproporphyrin after such treatments was, however, not found.

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 deficiencies 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-