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A Spectrometric Study of the Fluorescence Detection of Fecal Urobilinoids

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ABSTRACT: Fluorescence spectra of extracts of fecal material, in the presence of zinc ion, display the well-known green fluorescence used in the Schlesinger test for the urobilinoids, together with several other fluorescences. All of these can be demonstrated in a single spectrum by the synchronous fluorescence technique, which, with new extraction conditions, enables the urobilinoid fluorescence to be detected in quantities of human fecal material down to about 50 ng in mass. This represents a reduction on the order of a thousandfold in the detection limit of the original visual technique; selectivity too is greatly increased. The effects caused by a variety of reagents on the fluorescences have been examined. It is shown that in Edelman's version of the test the presence of mercuric ion largely quenches the fluorescence. The synchronous spectra vary considerably between different samples of human feces and between different mammals according to their diet. The specificity of the recommended technique is discussed with reference to the spectra given by other body materials.

KEYWORDS: criminalistics, luminescence, spectroscopic analysis, fluorescence spectrometry, fecal material

The techniques used in the characterization of fecal traces in forensic science include Schlesinger's [*1*], in which a green fluorescence is seen when an extract is treated with a solution of zinc acetate, and Edelman's [*2*], in which an additional treatment with mercuric ion produces a pink color.

A number of related compounds are thus detected. These include stercobilin and several urobilins [*3*], collectively referred to here as urobilinoids. These compounds are formed by bacterial processes in the gut from the bilirubin and its conjugates present in the bile. The major bacterial end-products are the colorless chromogens (stercobilinogens and urobilinogens) that in the presence of air, for example, are readily oxidized to the urobilinoids. Recent structural assignments for some of the compounds, all of which are members of the bilene group of the tetrapyrroles, are given by Brockman [*4*].

Although the Schlesinger and Edelman tests were originally devised for the clinical examination of both urine and fecal material, in forensic science work the main adaptation has been to the detection of fecal stains on swabs and clothing [*5*], on which only small amounts of material may be available. In this circumstance it is our experience that uncertain results are often obtained. Known fecal material sometimes gives no visible fluorescence, the fluorescence varies in color between samples, and the fluorescence may be entirely obscured by

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that of extraneous substances. We have therefore made a spectrometric study of these tests with a view to increasing their reliability and selectivity.

Experimental Procedure

The spectra were plotted with a Perkin-Elmer MPF-4 fluorescence spectrometer. The 5-mm square sample cuvettes were fitted with inlet and outlet lines for the transfer of samples and for the deoxygenation of the solutions when required. All of the spectra shown, except those in Fig. 1 (which are fully corrected), were run in a "ratio" mode with direct reference of the excitation response to the output of the xenon source at the relevant wavelength. Fully corrected spectra cannot always be obtained because the spectral range of interest extends beyond the limit of the usual rhodamine quantum counters.

Methanol, 2-methoxyethanol, and cyclohexane were bought as spectrometric-grade solvents. The methanol usually was adequately free of fluorescing impurities for use as received. Compounds fluorescing strongly in the 300-nm region were present in the methoxyethanol. They were eliminated when the solvent (in which the absence of peroxides was first confirmed [6]) was passed through a column of activated charcoal and rapidly distilled under nitrogen. Cyclohexane was treated similarly. Other materials were of analytical reagent grade. All samples were dried under vacuum, over silica gel, before examination.

The recommended procedure for the detection of urobilinoids and associated compounds is as follows. In 100 g of the methoxyethanol dissolve 1 g of zinc acetate dihydrate and 0.2 g of tris(hydroxymethyl)aminomethane (Tris). The absorbance of this reagent should not be greater than 0.04 per centimetre of pathlength, compared with distilled water, in the range of 270 to 700 nm, and 0.1 per centimetre of pathlength at 250 nm. The reagent is stable provided it is stored in darkness to prevent peroxide formation.

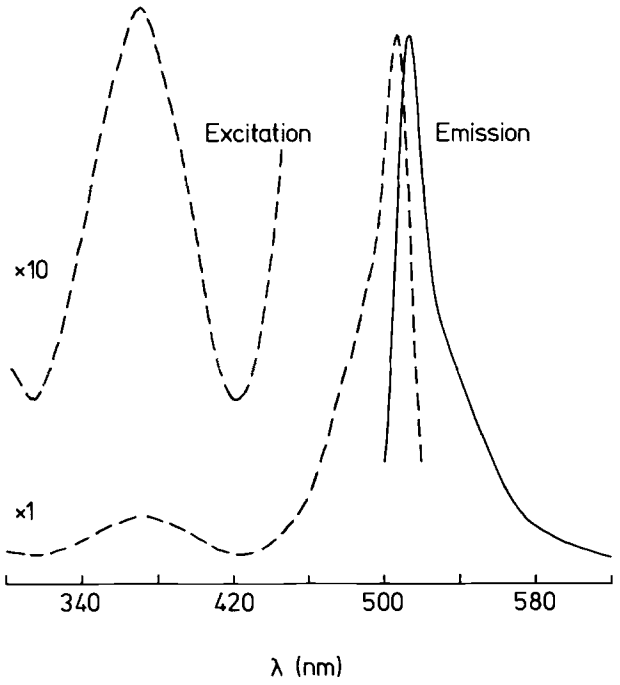

FIG. 1—*Fluorescence excitation (broken line) and emission (full line) spectra, corrected, of fecal urobilinoids in the zinc acetate reagent described under "Experimental Procedure."*

Coherent samples should be dried under vacuum and analyzed directly. Traces on (dried) swabs or fabric, for example, are removed with their supporting material and rinsed with cyclohexane. Retain the rinsings if the presence of mineral oil or other lubricants is to be sought, for example by fluorescence spectrometry [*7*] or gas chromatography [*8*]. To the sample add 1 mL of the zinc acetate reagent, sonicate for 5 min, heat at 100°C for 10 min, cool, and centrifuge. Plot the synchronous fluorescence excitation spectrum [*9*] of the supernatant at an excitation-emission interval of 6.5 nm over the range 250 to 700 nm, with bandpass settings not greater than 4 nm. When possible the spectrometer should record either an excitation-corrected spectrum or the ratio of the fluorescence to the output of the source at the corresponding excitation wavelength. If a peak is revealed in the region 507 ± 2 nm, plot its fixed excitation and emission spectra over the range of 300 to 600 nm. The presence of urobilinoids is indicated by excitation and emission maxima at 507 ± 2 nm and 513.5 ± 2 nm, respectively, as shown in Fig. 1. Note that if an uncorrected spectrometer is used, the excitation spectrum may exhibit a spurious fine structure caused by the spectrum of the xenon arc.

Strongly colored solutions should be diluted to minimize inner filter effects. The absence of a significant interference of this sort in the 250- to 300-nm range, where an important fluorescence occurs, can be shown by a comparison of the Rayleigh scattering signal (obtained with the excitation and emission monochromators set to the same wavelength) of the solution in this region with that from a reagent blank: the signal should be negligibly diminished. This is on the assumption that the absorbance of the reagent is as specified above, that no particulate matter is present, and that the contribution of fluorescence to the observed signal is relatively small, which it normally is here.

Results and Discussion

Fluorescence Spectral Characteristics of the Fecal Extracts

Shown in Fig. 1 are the corrected excitation and emission spectra of the fluorescence attributed to the urobilinoids extracted from human feces with the zinc acetate reagent. The excitation and emission maxima are at 507 and 513.5 nm, respectively. An additional weak excitation is present at 373 nm. With allowance for solvent effects and for the varying characteristics of fluorescence spectrometers, these results are consistent with others that have been published [*10–12*], although there is an as yet unexplained exception [*13*].

It is immediately apparent from Fig. 1 that the use of the usual long-wave (364-nm) ultraviolet lamps for the visual detection of this fluorescence is bound to give rise to difficulties. At 364 nm other fluorophores with strong excitations in this region will interfere. An example from case work is shown in Fig. 2. An extract of the suspected fecal material under an ultraviolet lamp gave a brilliant blue-white fluorescence, which was probably due to a detergent-derived fluorescent brightener on the garment concerned. The spectrum of this fluorescence, with a maximum at 440 nm (excited at 364 nm), is shown in Fig. 2. Any urobilinoid fluorescence here is entirely swamped. However, its presence is clearly demonstrated when an excitation at 500 nm is used; the corresponding excitation spectrum monitored at 520 nm is also readily obtained, as Fig. 2 shows. The excitation and emission wavelengths used here are slightly offset from their maxima because of the considerable overlap of the spectra.

Synchronous Spectrometry

By this technique several different fluorescences may be placed alongside one another in a single spectrum [*9*]. Figure 3 shows spectra of the fluorescence from two different samples of human feces, run with a synchronous excitation interval of 6.5 nm. This particular interval

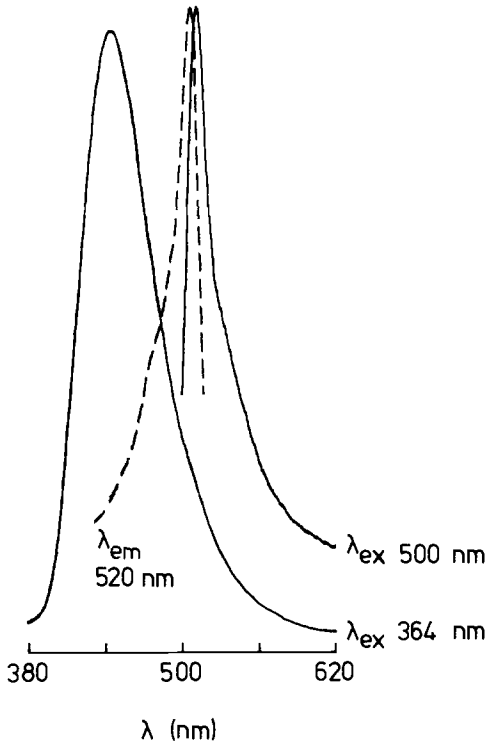

FIG. 2—*Fluorescence excitation (broken line) and emission (full line) spectra of a fecal stain on a clothing exhibit extracted into a zinc acetate reagent. Different fluorescences are excited at 364 nm and 500 nm, as described in the text.*

corresponds to the separation of the urobilinoid excitation and emission maxima and therefore maximizes the derived synchronous peak [*14*]. Apart from the characteristic urobilinoid peak at 507 nm, several other fluorescences are revealed. An examination of their excitation and emission spectra shows that these fluorescences are apparently due to chlorophyll-a at 657 nm, to compounds of the bilidiene group as their zinc complexes at 628 nm, to zinc-complexed porphyrins at 579 nm, and to aromatic amino acids at 285 nm. A variety of other fluorescences, not represented in these samples, sporadically occur.

The chlorophyll assignment is based on the close agreement obtained with published spectra and with the spectra of plant extracts in the zinc acetate reagent. For the bilidienes, which are derived from the urobilinoids by further oxidation, the small amount of published work is in agreement [*12*]. The characteristics of the 579-nm fluorescence agree with the fluorescence of protoporphyrin IX in this reagent, although uroporphyrin I and coproporphyrins I and II are similar and would be poorly resolved under these conditions (evidently, all of them are converted into their zinc complexes). The amino acid attribution is also based on direct spectral comparisons with the known compounds. It should be noted that the quoted synchronous fluorescence wavelengths are excitation wavelengths.

In human feces these other fluorescences are generally much weaker than the urobilinoid fluorescence and require higher spectrometric sensitivities to display them, as is shown for the region above 560 nm in Fig. 3. Even so, their varying amounts considerably affect the appearance of the extracts in ultraviolet light—the solutions used for Fig. 3 appeared quite different from one another—and limit the validity of visually based identifications.

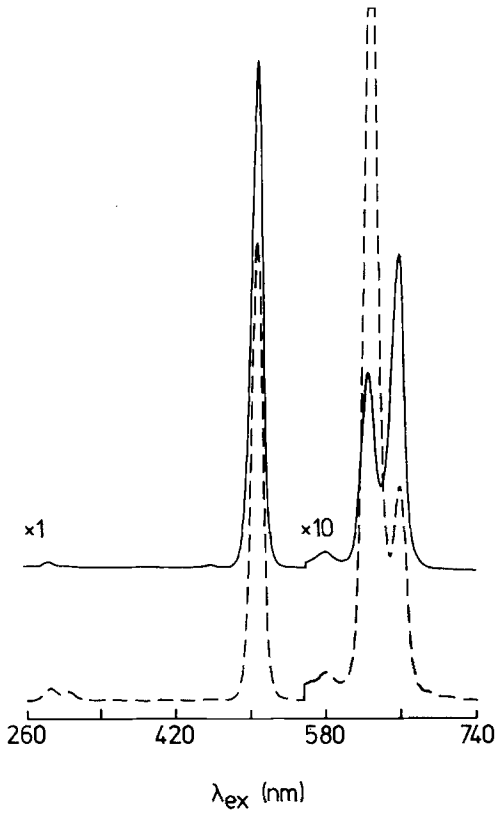

FIG. 3—*Synchronous spectra obtained with an excitation-emission interval of 6.5 nm from different samples of human fecal material. Further details are given under "Experimental Procedure."*

Reagent Composition

Conventionally, the Schlesinger reagent is an alcoholic solution of zinc acetate to which an extract of the questioned material is added. We have examined the effect on the intensity and wavelength of the fluorescence of a variety of modifications to the reagent. These include several alcohols, acetone, trimethylphosphate, chloroform, and acetonitrile as solvent components, and the addition of acetic acid, sodium acetate, water, Tris, pyridine, piperidine, and ammonium hydroxide in varying amounts. In each instance, 25 μL of a methanolic fecal extract (1 mg/mL) was added to 2 mL of the reagent containing 1% mass/volume zinc acetate dihydrate. The effects observed were relatively small: the highest intensities ($\sim$1.2 relative to methanolic zinc acetate) were obtained from alcoholic solvents containing Tris (0.2%), and the lowest (intensity, 0.42) from alcoholic acetic acid (5%). The addition of water (2.5%) to methanol solutions suppressed the fluorescence (intensity, 0.89). At higher concentrations of water, hydrolysis and precipitation of the zinc acetate occurred, and the fluorescence virtually disappeared. The wavelength of the synchronous excitation maximum shifted down to 504 nm, from 507 nm, in the presence of 2*M* ammonium hydroxide in methanol, which was the largest shift observed. The fluorescence intensity of the solutions increased only slightly ($\sim$5%) when they were purged with nitrogen; that is, oxygen quenching is slight.

Preferably, from convenience and sensitivity considerations, the extraction and the fluo-

rescence development should be done in a single step. To this end, a variety of experiments were made in which constant amounts (0.5 mg) of dried fecal material were treated with 1-mL volumes of different extractants. Each extract was then diluted (25 μL to 1 mL) in methanolic zinc acetate before spectrometry. The following conclusions were drawn. First, water, an often-used extractant, is very inefficient. Only a twentieth of the fluorescence obtained by the final technique was extracted. Second, higher yields are obtained in dry alcoholic solvents. The yield increases if the temperature is raised and is therefore limited by the solvent's boiling point. For example, relative to an extraction at room temperature, at 100°C in methoxyethanol the fluorescence intensity increased by a factor of 2.4. Third, the presence of zinc acetate increases the yield. In methoxyethanol the addition of 1% mass/volume zinc acetate dihydrate produced an increase of 2.1. It seems that complexation with zinc ion increases the solubility of the urobilinoids considerably.

Obviously, the usual technique can be considerably improved in a single-step procedure. Some more detailed results pertinent to the procedure described above are given in Fig. 4, which shows the effects of variation in concentration of zinc acetate and Tris, and in the heating time at 100°C for a methoxyethanol reagent. In each experiment 1 mg of fecal material was extracted with 2 mL of the reagent, as described above, except for the varied factor. Evidently, little further extraction occurs after 10 min at 100°C, the effect of Tris on the ex-

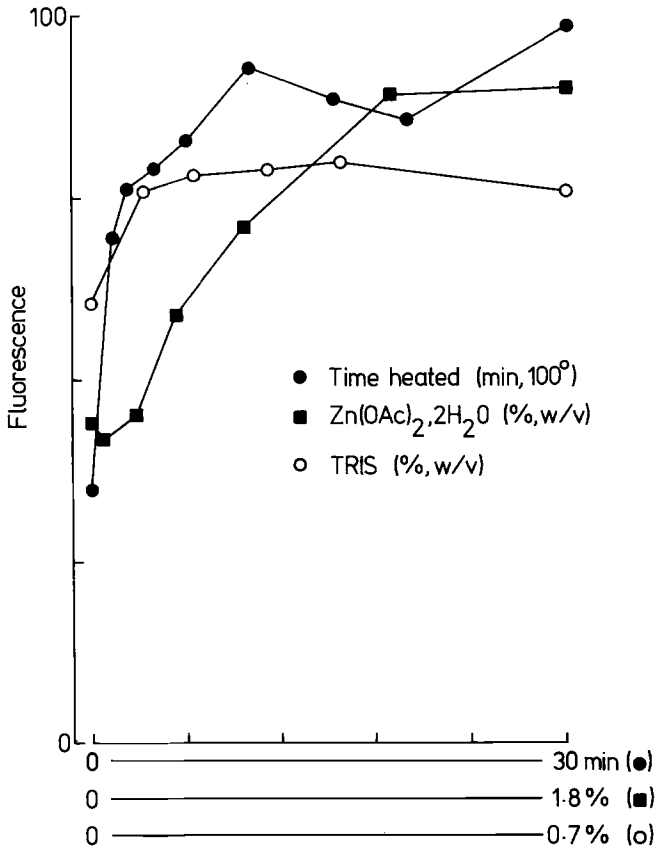

FIG. 4—*The effects of variation in experimental conditions on the urobilinoid fluorescence extracted from fecal material (0.5 mg/mL reagent). Apart from the varied factor, the conditions are as given under "Experimental Procedure."*

traction is small, and the optimum concentration of the zinc acetate is about 1% mass/volume. A significant fluorescence is detectable in the absence of zinc acetate, as Fig. 4 shows. Perhaps traces of zinc in the sample or solvent are responsible, although uncomplexed urobilinoids could be appreciably fluorescent [*11*]. Even under optimum conditions the extraction is incomplete. When a sample is re-extracted further amounts of urobilinoids are obtained ($\sim 10\%$ of the original), although not of the other fluorescent components.

The use of methoxyethanol as solvent is advantageous because of its high boiling point (120°C) and commercial availability in a relatively pure form. Fluorescent impurities may still be present, however, and must be removed if the short-wave fluorescence typical of body materials generally (see below) is to be determined.

Effects of Mercuric Ion and of Iodine

In Edelman's procedure [*2*], recommended by Nickolls [*5*] (who incorrectly ascribed the fluorescence to bilirubin), the sample is treated with a solution of a mercuric salt (mercuric chloride) to give a pink color before the addition of a zinc salt for the production of the fluorescence seen in the Schlesinger test. Unfortunately it seems to have remained unrecognized that the urobilinoids form very much more stable complexes with mercuric than with zinc ions. In the presence of mercuric ion only negligible amounts of the fluorescent zinc complex are formed. For example, when a fecal extract (1 mg/mL) containing 1% zinc acetate dihydrate was treated with the same concentration of mercuric acetate, the urobilinoid fluorescence was reduced in intensity by a factor of 0.03 and became, typically, visually undetectable (Fig. 5). The same result was obtained when the chloride salts were used. If substantially larger amounts of sample are taken, the fluorescence can be seen, but this differs to some extent from the characteristic color. Obviously, if both the pink mercuric complex of the urobilinoids and their fluorescent zinc complex are to be recognized readily in the same extract, the zinc salt must first be added and its effect determined. For more than 65 years the addition has been made the wrong way round! In any case, the mercuric coloration is generally weak and of little use for the small amounts of material to which the spectrometric technique is particularly applicable, and the distinctive fluorescence in the 620- to 660-nm region are largely suppressed also, as Fig. 5 shows.

In some applications of the Schlesinger test the fluorescence is enhanced by the use of iodine or persulfate, which produces more urobilinoids by the oxidation of the urobilinogens. With typical forensic science samples, which generally are dry and entirely aerated, the effect is fairly small. Presumably the oxidation is already practically complete. Figure 5 shows the effect of the addition of iodine at a concentration of 2×10^{-4} *M* to an extract of such a sample. The urobilinoid fluorescence is increased by a factor of 1.16. The bilidiene fluorescence at 628 nm, however, is largely destroyed. With increased concentrations of iodine the urobilinoid fluorescence rapidly decreases too. Apparently, more evidence could be lost than gained by this modification, as by Edelman's [*2*].

Detection Limits and Variation with Sample Size

Experiments with varying amounts of typical fecal material showed that the urobilinoid fluorescence could be detected, with a peak at three times the spectrometer's noise level, from 50 ng of material in 1 mL of reagent (estimated by dilution of more concentrated extracts). For the detection of the bilidiene and chlorophyll peaks at least 1 μg was required, and 50 to 100 μg was needed for the zinc-containing porphyrins. It was estimated that the limit of visual detection of the urobilinoid fluorescence in these solutions was in the range of 10 to 100 μg, a thousand times greater than that of the spectrometric technique.

Over a range of sample size from the detection limits to 2 mg in 1 mL reagent, taken in increments that increased by a factor of about 2, and taking $\log_{10}$ of each datum, the correla-

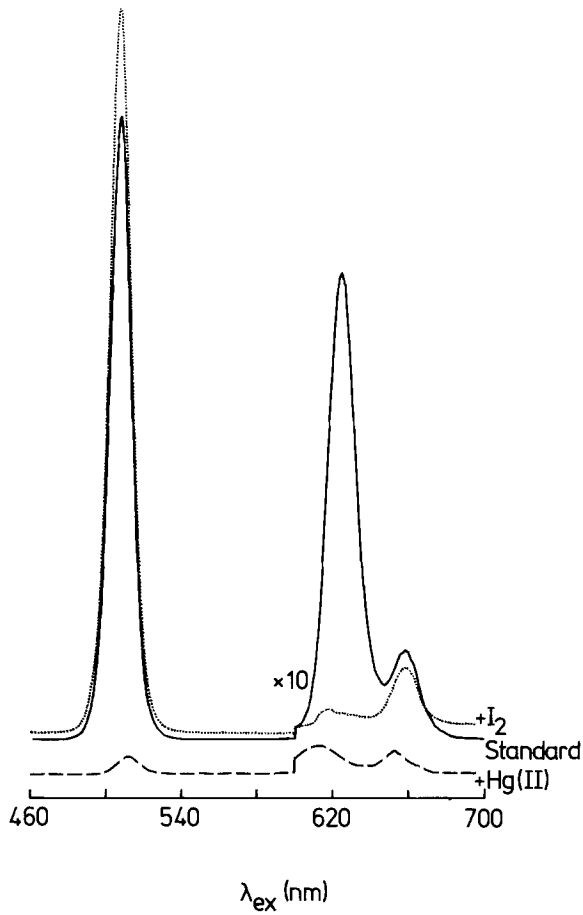

FIG. 5—*The effects of the addition of 0.0002* M *iodine (dotted line) and 1% mercuric acetate (broken line) on the synchronous fluorescence of a fecal extract (full line).*

tion coefficients of the $\log_{10}$ intensities on the $\log_{10}$ sample concentrations were 0.999, 0.995, 0.996, and 0.996 for the urobilinoid (19 points), zinc porphyrin (8 points), bilidiene (12 points), and chlorophyll (10 points) fluorescences, respectively. The respective linear regression coefficients of slope were 0.974, 1.024, 1.041, and 1.005.

The urobilinoid coefficient of slope differs significantly from the value of 1.0 expected for a direct linear dependence of intensity on concentration. This is thought to be mainly due to a decrease in the efficiency of the extraction as the sample size increases, although fluorescence quenching could also be involved. However, the effect on the appearance of the spectra is small and therefore has not been further investigated.

Variation Between Samples

Fecal materials vary widely in their urobilinoid content. In samples from 19 different individuals a fifteenfold variation in the urobilinoid fluorescence intensity, normalized with respect to sample mass, occurred. The other fluorescences varied to a similar extent, as shown for the two samples in Fig. 2, but these variations were found not to be greatly different from

those in a single individual sampled over a period of time. Evidently the spectra reflect the combined effects of an individual's diet, the bacterial flora of his or her gut, and the level of bilirubin excretion at a particular time. Within these limits the spectra could form useful evidence of origin of a transferred material.

Where the mass of material available for examination cannot be determined accurately, as is often the case, comparisons can be made only on the basis of relative intensities, such as those of the nonurobilinoid to the urobilinoid fluorescences. For the 19 different samples examined, a useful level of differentiation could be obtained in this way from 100- to 500-μg amounts. Thus, for the chlorophyll relative to the urobilinoid fluorescence intensity, on the assumption that a pair of samples was discriminated when the range of the combined results for the two samples, each run in duplicate, exceeded the upper 5% point of the relevant studentized range [*15*], the discriminating power [*16*] was 0.82. For the bilidienes the result was 0.70, and for these features taken together, 0.97. The zinc porphyrin fluorescences are generally too weak to be useful in this connection, although in some physiological states important amounts may be present.

Small samples do not yield spectra on which reliable differentiations can be based, although such samples may still be classified as being of fecal origin. Whereas samples of about 0.5 mg in mass, on cotton wool swabs stored in the laboratory atmosphere, have given results independent of time over a period of three months, in samples below the 50- to 100-μg region in mass the nonurobilinoid fluorescences generally become increasingly subject to the history of the sample and difficult to detect, in contrast to the urobilinoid fluorescence. Also, the typical heterogeneity of the material increasingly affects the results.

Relationship to Other Body Materials

Although the urobilinoids and their precursors are produced in the gut and are sometimes assumed, therefore, to be characteristic only of feces, they are to some extent absorbed into the blood stream from the gut and distributed throughout the body. The quantities involved are usually small but vary appreciably, particularly in hemolytic states. A useful measure of their relative amount can be made by the comparison of their synchronous fluorescence intensity with the intensity of the "amino" peak at 285 nm in the spectra. In fecal samples the latter fluorescence is generally only a small fraction of the former, as in Fig. 3, for example. In other materials the relationship is reversed, as shown for the two urine samples in Fig. 6. When 42 samples of urine were examined (10-μL aliquots were dried on filter paper and treated with the reagent directly), the urobilinoid fluorescence, relative to the amino peak, varied in 41 of the samples from less than 0.0014 up to 0.045. In the other sample the ratio was 0.161. (It should be noted that these ratios will vary with the spectrometer used to measure them.) In none of these samples was any significant fluorescence detected in the region above 600 nm. Similar results were given by other materials (vaginal fluid, semen, sweat, saliva) except blood. This gave a strong characteristic synchronous fluorescence at 645 nm and another at 525 nm that overlapped the urobilinoid peak. Figure 6 includes an example.

Provided that the urobilinoid and the other fluorescences are comparable in relative intensities to those shown in Fig. 3, for example, there is little possibility of confusion between human fecal material and any other human body material. When only traces are present, however, the bilidiene and chlorophyll fluorescences may not be detectable, or the latter may be spuriously present, and other materials may increase the 285 nm fluorescence. For instance, both urine and fecal material may be present on penile swabs in cases involving buggery. In this circumstance the evidentiary significance of the result will depend on whether the ratio of the urobilinoid to the amino fluorescence exceeds the value expected of urine. Ideally, this should be assessed on the basis of a sample of the defendant's urine.

Spectra from two cases in which swabs of this type were submitted are shown in Fig. 7.

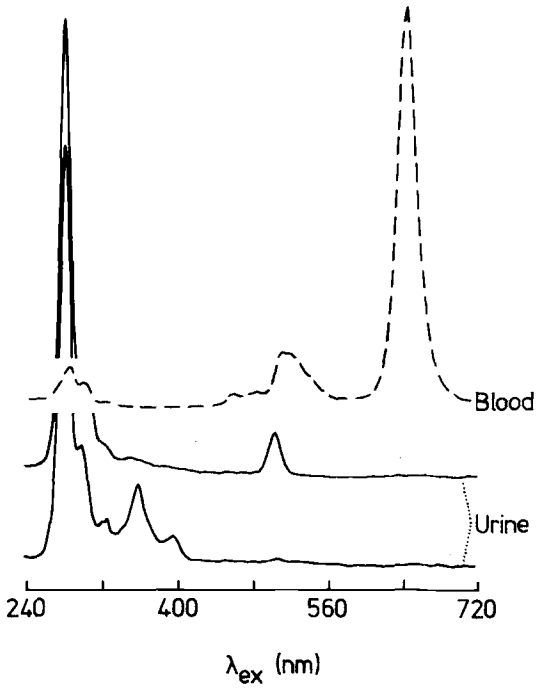

FIG. 6—*Synchronous fluorescence of a blood sample and two urine samples: 10 μL of each sample was dried onto filter paper and extracted with the zinc acetate reagent.*

Spectrum A is from a penile swab carrying a brown material. The spectrum shows that the presence of urobilinoids is detectable, but only with a hundredfold increase in sensitivity relative to the amino peak. The presence of fecal material is therefore excluded. Fecal material from the complainant in the case contained typical levels of urobilinoids. In Spectrum B, the urobilinoid fluorescence considerably exceeds the relative level expected from urine: the probable presence of fecal material is indicated. The excitation and emission maxima of the synchronous peak at 507 nm were the same as those already quoted. It is an important experimental point here that such swabs should be rinsed in cyclohexane before examination to remove any traces of mineral oil that might increase the 285-nm fluorescence. In the blank Swab C (Fig. 7) some traces still remain, although these are detected only at much higher sensitivities than are used in this wavelength region for Spectra A and B.

Urobilinoids are not invariably present in substantial amounts in human fecal material. The intestinal bacteria do not become well established until a few months after birth, and they may be temporarily eliminated by antibiotics. Although the technique described here has detected traces of urobilinoids under such conditions, for example in meconium, other fluorescence and absorbance spectrometric techniques are available by which the presence of unmodified bilirubin and its conjugates in such samples may be specifically shown [*13*].

Other Mammalian Fecal Material

Samples of other common mammalian feces have been examined. The results are largely predictable from the diet of the animal concerned.

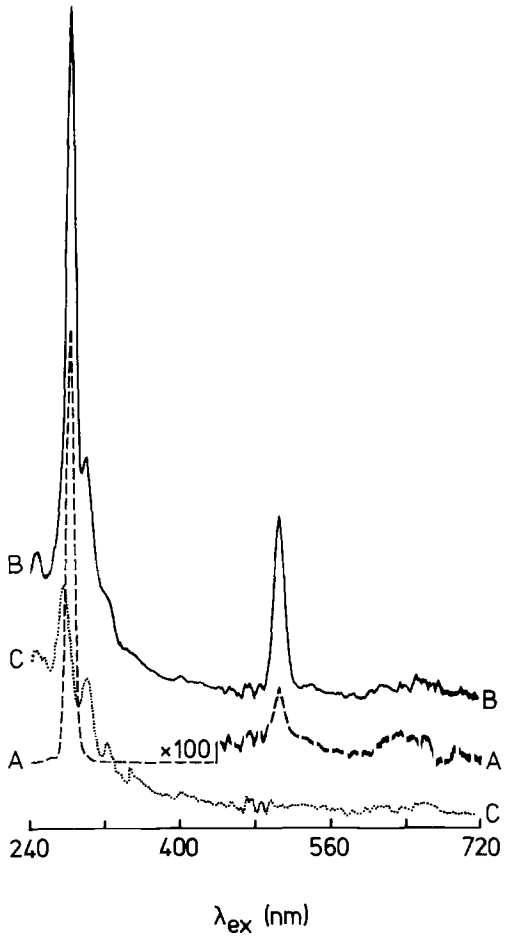

FIG. 7—*Synchronous fluorescence spectra from two penile swabs* A *and* B *and a blank swab* (C). *Spectrum C and the indicated longwave part of A were run at an increased sensitivity (×100).*

In canine fecal material (Fig. 8) the fluorescences detectable in human material are generally present, and in similar relative amounts, but a variety of others sometimes occurs, as the full-line spectrum shows. This could prove of value in the correlation of transferred traces, although the spectra do not in general differentiate less distinctive materials.

Other examples are shown in Fig. 9. In the porcine feces substantial amounts of urobilinoids are present but, relative to the amino peak, substantially less than in human material. The expected large amounts of unchanged chlorophyll in herbivore feces is shown in Fig. 9 for equine and bovine samples. There is also present in these a new synchronous fluorescence at 610 nm, the excitation spectrum of which was shown to be similar to that of chlorophyll. The compound responsible is assumed to be phylloerythrin, a chlorophyll catabolite. Urobilinoid fluorescence has always been found in such samples, but increased spectrometer sensitivities often must be used to detect it.

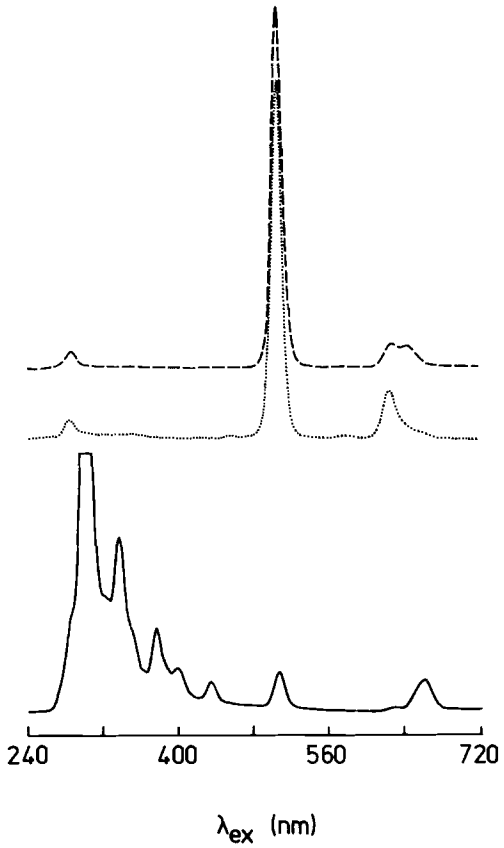

FIG. 8—*Synchronous spectra from three different samples (0.3 mg) of canine feces, extracted as under "Experimental Procedure."*

Concluding Remarks

Although the urobilinoids are commonly distributed, because the spectrometric technique enables their levels to be related to those of other fluorescent compounds, some of which are also typical, fecal traces may now be identified much more specifically than by the older technique, and at much higher levels of sensitivity. Of course, the results must be assessed in relation to those available from other techniques, particularly microscopy. But, in our experience, and as Nickolls pointed out [*5*], it is not uncommon for a sample to yield only sparse amounts of particulate matter, upon which a characterization by microscopy of the source of the sample can be based. Our technique may be readily and rapidly applied to such samples.

Recently an alkaline phosphatase test has been used for the characterization of mammalian feces [*17*]. No results for human feces were given, but an intestinal alkaline phosphatase is well known, and its relationship to the ABO and secretor genes has been studied [*18*]. Even though alkaline phosphatases are of widespread occurrence, if the reported detection limit (apparently, 85 μg) could be improved the results might usefully supplement those of the spectrometric technique.

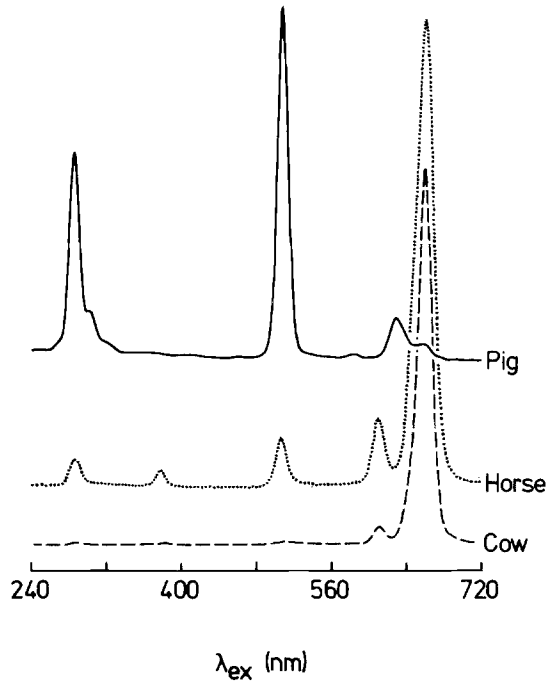

FIG. 9—*Synchronous spectra from extracts of 0.5-mg quantities of porcine, equine, and bovine feces. The conditions are as given under "Experimental Procedure."*

References

[1] Schlesinger, W., "The Clinical Identification of Urobilin," *Deutsche Medicinische Wochenschrift*, Vol. 29, No. 32, Aug. 1903, pp. 561-563.

[2] Edelman, A., "A Test for Urobilin in Urine and Feces for Clinical Purposes," *Wiener Klinische Wochenschrift*, Vol. 28, No. 36, 1915, pp. 978-979.

[3] Gray, C. H. and Howarth, P. J. N., *Clinical Chemical Pathology*, Edward Arnold Ltd., London, 1979, Chapter 6.

[4] Brockman, H., in *The Porphyrins*, D. D. Dolphin, Ed., Academic Press, New York, 1978, Chapter 9.

[5] Nickolls, L. C., *The Scientific Investigation of Crime*, Butterworth Ltd., London, 1956, p. 208.

[6] Vogel, A. I., *A Textbook of Practical Organic Chemistry*, 3d ed., Longman Ltd., London, 1974, p. 163.

[7] Lloyd, J. B. F., Evett, I. W., and Dubery, J. M., "Examination of Petroleum Products of High Relative Molecular Mass for Forensic Purposes by Synchronous Fluorescence Spectroscopy," *Journal of Forensic Sciences*, Vol. 25, No. 3, July 1980, pp. 589-602.

[8] Lloyd, J. B. F., "Capillary Column Gas Chromatography in the Examination of High Relative Molecular Mass Petroleum Products," *Journal of the Forensic Science Society*, in press.

[9] Lloyd, J. B. F., "Synchronized Excitation of Fluorescence Emission Spectra," *Nature Physical Science*, Vol. 231, No. 20, May 1971, pp. 64-65.

[10] Dhere, C. and Roche, J. "Research on the Fluorescence of the Urobilin Group of Pigments: Determination of Their Fluorescence Spectra," *Bulletin de la Société de Chimie Biologique*, Vol. 13, No. 9, Nov. 1931, pp. 987-1014.

[11] Bailenger, J., Faraggi, G., and Labadie, P., "Determination of Fecal Stercobilin," *Le Pharmacien Biologiste*, Vol. 8, No. 85, June-July 1973, pp. 285-292.

[12] Gray, C. H., in *Comprehensive Biochemistry*, Vol. 9, M. Florkin and E. H. Stotz, Eds., Elsevier Publishing Co., New York, 1963, Chapter 4.

[13] Roth, M., in *Clinical Biochemistry, Principles and Methods*, Vol. 2, H. C. Curtius and M. Roth, Eds., Walter de Gruyter, New York, 1974, Chapter 12.

[14] Lloyd, J. B. F. and Evett, I. W., "Prediction of Peak Wavelengths and Intensities in Synchronously Excited Fluorescence Emission Spectra," *Analytical Chemistry*, Vol. 49, No. 12, Oct. 1977, pp. 1710-1715.

[15] Pearson, E. S. and Hartley, H. O., *Biometrika Tables for Statisticians*, Vol. 1, Cambridge University Press, Cambridge, England, 1966, Table 29.

[16] Jones, D. A., "Blood Samples: Probability of Discrimination," *Journal of the Forensic Science Society*, Vol. 12, No. 2, April 1972, pp. 355-359.

[17] Thrasher, J. J. and Gecan, J. S., "Chemical Test for Mammalian Feces: Collaborative Study," *Journal of the Association of Official Analytical Chemists*, Vol. 64, No. 1, Jan. 1981, pp. 196-198.

[18] Boorman, K. E., Dodd, B. E., and Lincoln, P. J., *Blood Group Serology*, 5th ed., Churchill Livingstone, New York, 1977, pp. 384-386.

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