association ratios, and a higher, though still normally constant, proportion of mono-'oleo' disaturated glycerides.

We wish to express our grateful thanks to Prof. V. Subrahmanyam for his encouragement during the progress of these investigations.

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Indigoid Pigments Derived from a Pathological Urine

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WITH AN ADDENDUM ON

The Spectral Absorption of the Pigments

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This communication describes the isolation and identification of the indigoid pigments indirubin and indigotin from the acid-treated urine of a case of sprue. A relation between pigment production, severity of symptoms and the level of protein intake in the diet was observed.

MATERIAL

In the course of a study supervised by Wing-Commander T. F. Macrae of the urinary riboflavin excretion of cases of sprue among R.A.F. personnel, it was noticed that after autoclaving the urine for 15 min. at 120 lb. pressure in the presence of dilute hydrochloric acid (3.25 ml. of 2N-HCl to 25 ml. urine), adjusting to pH 4.0 and then shaking with chloroform, this solvent was occasionally coloured deep purplish red. A patient was given an experimental diet, rich in protein, for a preliminary period of 3 days and then for an experimental period of 5 days. The urine was collected during the experimental period, that is from the 4th to the 8th day after the commencement of the protein-rich diet. During the period of collection of urine the

quantity of urinary pigment extractable by chloroform after autoclaving increased markedly and at the same time the patient's condition deteriorated. Specimens of the pigmented chloroform extracts were submitted to me for an opinion concerning the nature of the colouring matter, and this formed the starting point of the present investigation.

Out of ten patients given the experimental diet, the urines of at least five yielded the red chloroform-soluble pigment. It cannot be stated with certainty that any member of the group was negative in this respect, since the phenomenon in question was only observed incidentally and was not a primary object of the investigation.

METHODS AND RESULTS

Spectral characteristics and quantitative determination of the crude pigment. The colour of the washed and filtered crude chloroform extracts was a reddish purple. The spectral absorption in the visible region was determined by means of the Pulfrich step-photometer, with the set of Zeiss filters supplied with the instrument. A marked absorption band

was found in the region of wave-length 530 m μ the extinctions being maximal with the green filter S 53 (see Fig. 1). This filter was accordingly used to

Fig. 1. Spectral absorption (step-photometer) of (a) chloroform extract of treated urine and (b) urine after extraction.

compare the quantities of pigment in the chloroform extracts of 25 ml. amounts of the treated urines from the commencement of high protein feeding until the 8th day; the results are presented in Table 1. Extinctions were measured in a 1 cm. cell, the chloroform extracts having all been made up to the same volume.

Table 1. Yield of pigment from urine of sprue patient receiving a high protein diet

Days on diet	Extinction using filter S 53	Urine vol. (ml.)	Relative yield of pigment
4	0.27	1790	1.00
5	0.28	1650	0.96
6	0.72	1465	2.18
7	1.14	1000	2.36
8	0.89	720	1.33

The diet during this period consisted mainly of a variety of dehydrated foods of which skimmed milk powder was the most important ingredient; some dried beef, dried egg and a comparatively small

amount of cereal products were the principal remaining ingredients. The diet supplied about 3300 kg.cal. daily, had a fairly normal fat content (about 100 g. daily) and was rich in protein (140 g. daily).

For the first few days after institution of the diet only a trace of pigment was recoverable from the chloroform extract of the autoclaved urine; the amount increased markedly on about the 4th day. It would appear that there was in this case a definite relation between the level of protein intake and excretion of chromogen.

Isolation of indigotin and indirubin. The pigment present in the chloroform extracts could not be removed by shaking with 25% (w/v) HCl or NaOH. Upon evaporation of the solvent, it was left as a soft, amorphous mass which was insoluble in cold light petroleum but moderately soluble in hot benzene, from which it showed some tendency to separate in crystalline form. Petroleum removed a small quantity of colourless material.

After washing the pigmented residue from the pooled chloroform extracts repeatedly with cold petroleum, it was dissolved in a small quantity of hot benzene and passed through a column of adsorbent alumina (Savory & Moore) and the chromatogram developed with benzene. There appeared (a) a rapidly descending eau-de-nil-coloured zone which was completely eluted from the column and collected; (b) an intensely coloured cerise red band comprising the major quantity of the pigment and which separated only slowly from (c) a smaller uppermost layer of chocolate brown material: (b) and (c) were obtained by mechanical division of the column after a sufficient separation between their boundaries had been accomplished by development and the pigment of zone (b) was removed from the alumina by repeated washing with hot chloroform.

The solution so obtained had a bright cerise colour and exhibited the absorption spectrum shown in Fig. 2 (Zeiss step-photometer). Evaporation of the solvent left a crystalline residue which was dissolved in a little hot benzene. On cooling, the deep red solution deposited the greater part of the pigment in flattened needles or laths frequently aggregated into rosettes. This material agreed in properties with indirubin or indigo red, the isomer of indigo blue. Its identity with indirubin was confirmed by step-photometric comparison with an authentic specimen of the latter (for chromatographic examination see p. 674) for which I am indebted to Prof. A. Rowe, of Leeds, with both benzene and chloroform as solvents (see Figs. 2, 3). Detailed comparison of the spectral absorption in the ultra-violet and visible regions of the synthetic and natural materials confirmed this identification (see Addendum and Fig. 6).

The blue pigment (zone *a*) which was eluted from the chromatographic column afforded, on concentration of the benzene, a deep blue solution with a tinge of purple. Upon evaporation of the solvent it separated in crystalline form and was recrystallized from benzene affording dark blue overlapping

Fig. 2. Spectral absorption (step-photometer) in chloroform of (○) purified red pigment from urine and (×) of synthetic indirubin.

plates or laminae. This pigment was identified with indigotin by measurement of its absorption bands in different solvents using the Hartridge reversion spectroscop and comparison with an authentic specimen of indigo (see Table 2). The spectral absorption in benzene, measured with the step-photometer, was also identical with that of indigotin (see Fig. 4).

Table 2. Comparison of blue pigment with indigotin using different solvents and Hartridge reversion spectroscop

Solvent	Colour of solution	Position of absorption maxima	
		Indigotin (mμ)	Blue pigment (mμ)
Benzene	Purplish blue	598.8	599.6
Chloroform	Blue	601.5	601.0
Aniline	Green	630.2	630.0

In the crude chloroform extract of the treated urine the ratio of indirubin to indigotin was approximately 2:1.

Fig. 3. Spectral absorption (step-photometer) in benzene of (○) purified red pigment from urine and (×) of synthetic indirubin.

Fig. 4. Spectral absorption (step-photometer) in benzene of (○) purified blue pigment from urine and (×) of indigotin.

DISCUSSION

Presence of an indirubin chromogen in urine in other conditions. Indigoid pigments have been obtained from normal and pathological urines in a variety of conditions and by numerous authors. In most cases, however, the identification of the pigments has not been sufficiently thorough. The familiar Jaffe and Obermayer reactions for indican (potassium indoxyl sulphate) both depend, of course, upon the production of indigo from indoxyl under the influence of acid and an oxidizing agent, and Rosin (1891) noted that some indirubin was produced in these reactions together with indigotin, especially if the temperature was raised. Rosin also isolated and satisfactorily identified indirubin from horses' urine treated at boiling temperature with dilute nitric acid. He demonstrated its formation from pathological urines, particularly when the patient suffered from intestinal disorder or wasting disease, and obtained it also from some apparently normal individuals by the same process. In this remarkably thorough study he showed that for the formation of indigoid pigment decomposition and oxidation of indoxyl are essential, that the product could vary according to the conditions employed and also that when indigo is sublimed by heating, some conversion into the isomeric indirubin takes place. In natural indigo from the plant, some of the red isomer accompanies the blue. The structural relationship between indigotin (blue) and indirubin (red) will be clear from the accompanying formulae:

Indigotin (indigo blue)

Indirubin (indigo red)

Occasionally the indigoid pigments have been found preformed in urine, or at any rate their formation has taken place spontaneously and rapidly after the specimen was passed. Thus Rosin (1891) describes the separation on standing of a sediment containing both indigotin and indirubin from urine of a case of cystopyelitis, and Dorner (1922) found indigotin not only in the urinary sediment of a patient with kidney stones but also in the substance of the renal stones themselves. In these

cases the oxidizing agent necessary to transform the precursor (probably indoxyl) into pigment is presumably derived from the metabolizing bacteria.

Relation of indirubin formation to the urochrome reaction and other tests. There has been in the past considerable confusion between indirubin and other red pigments formed from urine as for example urochrome in Nencki & Sieber's (1882) urochrome test (N.S. test). The latter is carried out by adding to the urine a few drops of sodium nitrite solution and then 25 % (w/v) hydrochloric acid. The reaction has been studied by Herter (1908), Watson (1938, 1939) and others, and there seems little doubt that the pigment, probably nitrosoindole-acetic acid (Plimmer, 1920), is derived from indolylacetic acid as demonstrated by Herter (1908) or its conjugate with glycine, indolylacetic acid (Homer, 1915).

The Ellinger-Dojmi (1935) reaction was applied to the urine of pellagrins by Beckh, Ellinger & Spies (1937), who believed it to be a measure of porphyrin excretion but Watson (1938, 1939) showed that this was not the case. The B.E.S. test, as it has become known, is similar to the N.S. test, and the pigment produced when the ether extract of the urine is shaken with 25 % (w/v) hydrochloric acid is urochrome. Watson with his collaborators (e.g. Watson & Layne, 1943) has made a careful study of the N.S. and B.E.S. tests and their relation to a red pigment which he observed in the preservative layer of toluene in contact with urines of pellagrins and certain patients with malnutrition of one cause or another. From the coloured toluene layer, a red, crystalline pigment was separated 'closely related at least to indirubin'. No correlation could be found between the appearance of this pigment in the toluene and other evidences of nicotinic acid deficiency. Previously, Watson (1939) had stated that it appeared highly probable that the pigment was indirubin and concluded that there existed a close relationship between the production of this pigment and the urochrome reaction. He observed in certain urines that the B.E.S. test became negative as a red coloration developed in the toluene layer of the urines on standing. However, the outcome of the N.S. or B.E.S. tests did not always correspond with its formation, many urines which exhibited strongly positive tests failing entirely to develop a coloured toluene layer. Nitrite does not seem to be necessary for its formation.

Chemical and spectral characteristics of urochrome. Urochrome as formed in the N.S. and B.E.S. tests is a cherry-red pigment the colour of which is discharged by neutralization of the mineral acid. It is not extractable from the aqueous layer by ether or chloroform and is thus easily distinguishable from indirubin. It is, however, extractable by amyl alcohol in which it shows an absorption band at 555-557 m μ , but in 25 % (w/v) HCl solution two

weak bands appear at 544 and 511 $m\mu$ respectively (Watson, 1939). Homer (1915) gives 552–531 and 505–492 $m\mu$ (faint) for the pigment derived from indolyacetic acid and 579–539 and 502–485 $m\mu$ (very faint) for that from indolyaceturic acid, and demonstrated the ready conversion of the latter into the former when urine is allowed to stand.

Fig. 5. Spectral absorption (step-photometer) of urorosein. (○) Mixture of indolyacetic acid and HCl, (x) the same after shaking with chloroform.

Solutions of indolyacetic acid in 20% (w/v) hydrochloric acid turn red on standing, and such a solution when examined by the Pulfrich step-photometer was found to have a spectral absorption with evidence of a maximum at about 530 $m\mu$ (see Fig. 5). Similar ill-defined maxima were encountered in urorosein solutions prepared by the N.S. technique, but by shaking these acid solutions with chloroform a considerable quantity of brown pigment was removed, showing only general absorption, and leaving a clear pink colour in the aqueous phase. This pigment now exhibited a well-defined absorption spectrum with an intense maximum at about 530–540 $m\mu$ (filter S53) as shown in Fig. 5. There is a striking similarity between the form of the absorption curve of urorosein and that of indirubin in chloroform or benzene (Figs. 2, 3) which supports the hypothesis of a close structural relationship between the two substances. Whilst, however, the

precursor of urorosein in urine is almost undoubtedly indolyacetic acid or its conjugate, this substance did not give rise to any indirubin when autoclaved in acid solution under the same conditions that produced indirubin from the sprue urine. Ellinger (1903, 1913) has postulated that indirubin arises from the condensation of indoxyl with isatin produced from the latter by oxidation, thus:

Such a reaction is known to take place in alkaline ethanolic solution (Schunk & Marchlewski, 1895a, b), but this simple explanation of indirubin formation is not accepted by Maillard (1913) although it would seem to be a very reasonable suggestion. Maillard (1901) has pointed out that if oxidation of the indoxyl-like precursor of these pigments is carried out rapidly indigotin results, but if slowly, indirubin is the main product.

Fearon & Thompson (1930) describe another class of red pigments, namely, the urocarmines which are produced by union of indoxyl with phenols (having a free *p*-position) in the presence of oxidizing agents. They are not, however, chloroform soluble and are thus not likely to be confused with indirubin.

An absorption band in a position almost identical with that of urorosein from indolyaceturic acid is seen in the pigment 'skatole red' described by Brieger (1880), viz. 577–550 $m\mu$. Homer (1915) does not consider skatole red to be a single substance and also finds that the mixed pigments obtainable from urines following skatol administration are much more stable than urorosein.

Relation of Watson's toluene-soluble red pigment to the pigment from sprue urine. As stated above, Watson noted considerable similarity between the pigment found by him in the layer of toluene preservative added to pathological urines and indirubin, but identity was not established. Schwartz, Marvin, Layne & Watson (1943) attempted to settle this question by use of the chromatographic technique. The toluene-soluble pigment from human or dog urine was adsorbed on alumina and washed by petroleum containing increasing quantities of ethyl acetate. The human pigment yielded six

zones, one of which was blue-green and broad, another red-violet in colour. Canine urine gave no blue pigment but four zones, one of which was red and one violet. A specimen of synthetic indirubin (Pont de Nemours Co.), prepared from isatin and indoxyl, was also chromatographed but afforded no less than four zones, a purple, two violet and a blue. Ultra-violet absorption curves made from the violet pigments and sublimation temperatures were compared with those of the main human and canine fractions, and similarity but not identity demonstrated. These authors concluded that none of the pigment isolated from the urines was identical with synthetic indirubin.

It is rather difficult to understand this statement as 'synthetic indirubin' was found to be a mixture of several different pigments. The indirubin used for comparison in the present work was obtained from the chemical museum of the Department of Colour Chemistry, Leeds, and was well crystallized. When chromatographed in benzene solution on alumina it appeared homogeneous except for a trace of blue which rapidly passed out of the column (indigotin). Further development with 1:1 mixture of petroleum and ethyl acetate caused the separation of another trace of impurity, a very narrow pale mauve band moving more slowly than the indirubin. The main band after this treatment could not be further resolved even by chloroform as developing agent. It is quite clear that Watson and his collaborators were unlucky in having obtained a very impure specimen of material with which to compare their urinary pigments.

As proof of identity of the pigment from sprue urine here described with indirubin may be cited the fact, in addition to the spectroscopic evidence already presented, that when mixed and developed chromatographically on alumina no separation of these two pigments was found possible. It thus seems clear that the pigment in question was indirubin. Whether or not Watson's toluene-soluble pigment is in fact the same must remain an open question.

Relation of indirubin production to sprue and to the diet. From what has been stated, it will be clear that indirubin may be produced from many pathological urines, especially those from cases of malnutrition or intestinal disease. It cannot be considered specific for sprue, but is almost certainly related to the disordered digestive and absorptive processes of this condition. The finding that its production is increased on a high-protein diet supports the idea that its precursor is indoxyl or a similar product of tryptophan metabolism.

In this connexion it is interesting to note that Homer (1913) found no chromogens excreted in the urine after the administration of tryptophan in amounts as large as 10 g. to normal dogs on a diet of milk and bread; indolcarboxylic acid was eliminated in the unchanged state where in the urine it gave rise on oxidation to a typical pigment with absorption band 505–484 $m\mu$; indolaldehyde gave rise to urinary indican forming the chloroform-soluble blue pigment with absorption 627–598 $m\mu$. Homer concludes that her data point to the liver as being the site of decomposition of tryptophan, and that the presence of indoxyl compounds in the urine is not of necessity due to bacterial disturbances in the intestinal tract but may owe its origin to the formation of indolaldehyde in the liver as a result of the failure of the disordered liver cells to deal normally with tryptophan.

SUMMARY

1. The chemical characteristics and spectral absorption are described of a chloroform-soluble pigment obtained from the treated urine of a patient suffering from sprue. The urine was heated with dilute acid prior to extraction.

2. From the crude pigment, indirubin (indigo red) and indigotin (indigo blue) were isolated in an approximate relative proportion of 2:1. These pigments were crystallized and characterized by their spectral absorption.

3. The quantity of mixed pigment excreted daily by the patient was found to increase greatly when he was placed on a diet high in protein but normal in fat. Whilst he was on this diet his clinical condition deteriorated.

4. The relation is discussed of indirubin formation to the urorosein reaction (Nencki & Sieber and Beckh, Ellinger & Spies) and to the toluene-soluble red pigment observed by Watson to be present in certain pathological urines, particularly those of some pellagrins and patients with gastro-intestinal disease.

My thanks are due to Wing-Commander T. F. Macrae for having brought this pigment to my notice and to him and his assistants, Flight-Sergeant G. A. Childs and Sergeant S. Parkin, for securing material for the investigation. Prof. A. Rowe of the Department of Colour Chemistry, Leeds, kindly supplied the specimen of indirubin from his museum collection. I am also indebted to Miss H. Sheinman for technical assistance rendered during the course of the work. I wish to acknowledge receipt of a grant from the University of London Research Grant Fund which enabled me to purchase the Hartridge reversion spectroscope used in this work.

ADDENDUM

SPECTRAL ABSORPTION

BY E. R. HOLIDAY AND E. M. JOPE

From an examination of the spectral absorption, in the ultra-violet and visible, of synthetic indirubin (Rowe) purified chromatographically (p. 674) and that of the red pigment isolated from treated urine

(p. 670), the identification of these two pigments has been confirmed.

Their spectral absorption curves were obtained with a Hilger medium quartz spectrograph E498

Fig. 6. Comparison of spectral absorptions in chloroform of synthetic indirubin (full curve —) and red pigment isolated from treated urine (points ···) both solutions $0.0805 \times 10^{-3} \text{ M}$. The full curve for synthetic indirubin is based upon twice as many experimental points as those shown for the natural product. The fine structure band systems of these absorption spectra and of that of indigotin in chloroform are shown diagrammatically.

$$\epsilon_{\text{mol.}} = \log_{10} \frac{I_0}{I} \times \frac{1}{cd}$$

and Spekker photometer, with a tungsten steel spark as a light source with Ilford process plates for the wave-length range 230–400 $m\mu$, and a tungsten ribbon filament lamp with Kodak IIIF plates for the range 400–690 $m\mu$. The spectrograms were matched on a photoelectric microphotometer.

The spectral absorption curves of the synthetic and natural products in chloroform agreed closely with one another over the whole range 230–690 $m\mu$ (Fig. 6). Further examination of these pigments by a modification of the technique of Holiday (1937) revealed the existence of fine structure band systems in their ultra-violet spectral absorption, and the identity of position of these bands for the two pigments makes their chemical identity much more certain than would the mere demonstration of a close similarity between the envelopes of their absorption curves (Holiday, 1945). By dissolving first in a drop of chloroform it was possible to get sufficient indirubin into hexane for examination, and with both synthetic and natural products a system of seven bands was observed similar to that in chloroform but as a whole displaced by 5 $m\mu$ to the shorter wave-lengths. Woodward (1941) notes an average displacement of 7 $m\mu$ between the wave-lengths observed in these two solvents of the intense absorption peak at $\lambda=220$ –260 $m\mu$ of a large number of $\alpha:\beta$ -unsaturated ketones. The use of hexane did not in the case of indirubin give sharper fine structure than chloroform. The fine-structure band systems of indigotin isolated from the same urine (p. 670) were also observed in chloroform solution and are recorded in Fig. 6 for comparison.

Table 1. Wave-lengths and molar extinction coefficients of the absorption peaks of indirubin in chloroform

Present data		Data of Cholewinski & Marchlewski (1938)	
λ ($m\mu$)	ϵ_{mol}	λ ($m\mu$)	ϵ_{mol}
242	17,200	—	—
291.5	22,000	289.5	20,900
362	4,600	360	5,300
540	10,400	—	—

The spectral absorption data for indirubin presented here may be compared with that of Cholewinski & Marchlewski (1938) (see Table 1) who used similar equipment, but matched their spectrograms

visually. Attention may be called to two errors in their paper: (1) from the tables of experimental data (their Table 2) it is clear that the concentration of indirubin used must have been 0.0001M and not 0.00001M as stated; (2) $\log \epsilon_{\text{mol}}$ for the 360 $m\mu$ band in chloroform is 3.73 as shown in their Fig. 1 and not 3.23 as appears in their Table 2. They give no details of purification of their specimens. They do not record the visible band, and their absorption curves give no hint of the fine structure detected in the present study.

Observation of fine structure in the low-intensity bands of indirubin and indigotin in the region $\lambda=350$ $m\mu$, and similar unpublished observations (Jope & Jope) in the corresponding bands in the region $\lambda=320$ $m\mu$ of acetophenone and many other compounds containing the group

present also in these pigments, indicates that the electron transition causing this band, although probably located in the C=O group, is coupled to the vibrational energy levels of the benzene ring to which that group is conjugated. This band with its superimposed fine structure may be considered as characteristic of the benzoyl group acting as a chromophore. This evidence supports the similar interpretation of Morton & Stubbs (1940).

The intense absorption bands in the visible of indirubin and indigotin in organic solvents must be due to the whole molecule acting as a chromophore in each case, and this is explained by van Alphen (1941), Sheppard & Newsome (1942) and others as being due to a resonance between two structures, e.g.,

some unknown factor favouring the o-quinonoid form. Sheppard & Newsome explain the variations in spectral absorption in the visible and hence the colours of indigotin solutions in different solvents on the basis of the above resonance hypothesis.

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Combination between different Proteins and between Proteins and Yeast Nucleic Acid

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There are many substances that reversibly inhibit the infectivity of tobacco mosaic virus. The mechanism of inhibition is largely unknown, but some of the most effective inhibitors, trypsin and ribonuclease for example, combine with the virus (Kleczkowski, 1944; Loring, 1942). Other substances, such as clupein and nicotine, also combine with the virus (Bawden & Pirie, 1937a, b), though their effects on infectivity have not been tested. Combination between the virus and ribonuclease or clupein is apparent from the formation of visible precipitates when salt-free solutions of the components are mixed. The precipitates dissolve when neutral salt is added.

Precipitation of proteins by protamines, and of nucleic acids by proteins or protamines, is a well-known phenomenon that has been extensively studied (Ugglas, 1914; Steudel & Peiser, 1922; Hammarsten, 1924; Lissitzin & Alexandrowskaja, 1933). It is generally accepted to be the result of a salt-like combination occurring between oppositely charged substances. Similar phenomena have not been hitherto observed with pairs of proteins both having isoelectric points in the acid or in the alkaline region, and in the main they have also been confined to combinations between pairs of substances in solution, though it has been shown that suspensions of heat-coagulated egg albumin can combine with dissolved yeast nucleic acid (Przylecki & Grynberg, 1932), and also that solid yeast nucleic acid can combine with dissolved amylases (Butler, 1945). In the present paper it is shown that insoluble heat-coagulated proteins behave like proteins in solution and can combine with dissolved

proteins, clupein and yeast nucleic acid. The conditions determining such combinations have been studied and attempts have been made to measure the effects of such combinations on the infectivity of tobacco mosaic virus.

MATERIAL AND METHODS

Purified tobacco mosaic virus, prepared by the method described by Bawden & Pirie (1943), was used.

Crude serum globulin was obtained from human serum by half-saturation with $(\text{NH}_4)_2\text{SO}_4$; the resulting precipitate was filtered off, washed in half-saturated $(\text{NH}_4)_2\text{SO}_4$, dissolved in H_2O and dialyzed against 0.9% NaCl.

Crude serum albumin was prepared from horse serum. After removing the globulin fraction by half-saturation with $(\text{NH}_4)_2\text{SO}_4$ and filtering, the albumin was precipitated by saturating the filtrate with $(\text{NH}_4)_2\text{SO}_4$. The precipitate was filtered off, dissolved in H_2O and dialyzed against H_2O .

Globin was prepared from rabbit haemoglobin. Blood corpuscles were sedimented from oxalated rabbit blood by centrifugation, washed several times with 0.9% NaCl and haemolyzed by adding distilled water. All sedimentable material was removed by centrifugation. Globin was prepared from the resulting solution by the HCl-acetone method of Anson & Mirsky (1930). The precipitate was washed thoroughly with acetone until washings were colourless, and dried.

Crystalline ribonuclease was prepared from beef pancreas by the method described by Kunitz (1940). The enzyme was twice crystallized from $(\text{NH}_4)_2\text{SO}_4$ solution, dialyzed against distilled water and dried.

Commercial preparations of clupein sulphate and of yeast nucleic acid were obtained from the British Drug Houses Ltd. About 40% of the nucleic acid was precipitated when 10 vol. of glacial acetic acid were added to 1 vol. of the neutral solution.